

ACNP 49th Annual Conference

Poster Abstracts

Poster Session I

December 6, 2010 5:30PM-7:30 PM

1. A Brain Microarray Study of Lithium-Treated Mice and Knockout Mice with Lithium-Like Behavior Reveals a Common Effect on Mitochondrial Function

Lilach Toker, Yuly Bersudsky, RH Belmaker, Galila Agam*

Ben Gurion University of the Negev, Beersheva, Israel

Background: The molecular mechanism of lithium (Li)'s mood-stabilizing action is not yet unraveled. Since its clinical efficacy is characterized by a lag in onset of 1-3 weeks, it is reasonable that the therapeutic effect requires reprogramming of gene expression. Previous studies have shown alteration in gene expression following mood stabilizing drug treatment in a variety of genes, including genes involved in inositol metabolism. Inositol-monophosphatase 1 (IMPAse1) is inhibited by therapeutically-relevant Li concentrations in an uncompetitive manner, possibly resulting in decreased inositol, subsequent down regulation of the phosphatidylinositol (PI) cycle and dampening of assumed hyperactive neurotransmission through this pathway (the "inositol depletion" hypothesis). Li was also shown to down-regulate the expression of sodium myo-inositol co-transporter (SMIT1), responsible for the uptake of myo-inositol from extracellular fluid. Both IMPA1 and SMIT1 homozygote knockout mice exhibit lithium-like behavior in the forced-swim test and the pilocarpine-induced seizures paradigm. We aimed to identify gene networks and pathways affected in homozygote IMPA1 and SMIT1 knockout mice and in Li-treated mice compared with wild-type (WT) untreated mice. Since our results of differentially expressed genes culminated in mitochondrial function, we used the oxidative phosphorylation inhibitor rotenone to evaluate behavioral reversal of Lithium effects.

Methods: For the microarray study male, 2 months old, IMPA1 and SMIT1 homozygote knockout mice and their littermate wildtype (WT) mice were used. Mice received powdered food. Li-treated WT mice received powdered food supplemented with 0.2% Li for five days, followed by 0.4% Li for another 10 days. Microarray analysis was performed using the Affymetrix platform. Results were analyzed using the softwares IPA (Ingenuity Pathway Analysis), GSEA (Gene Set Enrichment Analysis) and DAVID (Database for Annotation, Visualization and Integrated Discovery). For the rotenone-treatment study two groups of male, 2 months old, ICR mice were administered subcutaneously for four weeks either 0.5% DMSO in 0.9% NaCl (vehicle) or 0.5 mg/Kg rotenone in vehicle. At the end of the second week each of the groups was subdivided into two groups receiving either lithium food or regular food as described for the microarray study.

Results: We show that oxidative phosphorylation and mitochondrial function are the only significant pathways commonly affected in the frontal cortex of SMIT1 and IMPA1 knockout mice and Li-treated mice. Administration of rotenone, an inhibitor of mitochondrial oxidative phosphorylation, augments the hyperlocomotion response of mice to d-amphetamine, an effect attenuated by Li treatment.

Discussion: Our results corroborate previous finding in bipolar patients and suggest that improvement of mitochondrial dysfunction, mediated by inositol depletion, might underlie the therapeutic effect of Li.

Disclosure: L. Toker: None. Y. Bersudsky: None. R. Belmaker: None. G. Agam: None.

2. A Developmental Animal Model of ADHD: Drug-Seeking, Impulsivity and Sex Differences

Heather MacGillivray, Britta Thompson, Susan Andersen*

McLean Hospital/Harvard Medical School, Belmont, MA

Background: Attention deficit hyperactivity disorder (ADHD) is a developmental disorder wherein a subset of individuals with ADHD is at greater risk for substance use than the typical population. Substance use disorder (SUD) emerges earlier in the ADHD population. Impulsivity is an additional risk factor for addiction, and is another cardinal feature of ADHD. One measure of impulsive choice is delayed discounting and occurs in 26.8% of children with ADHD. We were interested in determining whether the same developmental manipulation could produce both a developmental shift in drug sensitivity and increase impulsivity. We have recently shown that sensitivity to cocaine-associated environments, a measure associated with drug-seeking in rats, is relatively low during the juvenile period and increases during adolescence, paralleling what is observed in humans. A shift in cocaine sensitivity during the juvenile stage would be consistent with enhanced risk for later SUD. In addition, increased delayed discounting would further enhance risk for SUD. While the underlying biochemistry of ADHD is poorly understood, reduced cortical dopamine levels have been implicated in a number of studies. In addition, little is known about how dopamine levels may differentially alter behavior in juvenile males and females.

Methods: Sprague-Dawley rat pups that were 10-11 days of age were pretreated with desipramine to protect noradrenergic terminals, anesthetized with ice hypothermia, and given a bilateral, intra-prefrontal microinjection of 6-OHDA or vehicle. Previous studies have shown that 6-OHDA does not produce a total depletion of dopamine when given during development. Subjects were then allowed to mature to weanling age of 22 days of age. Juvenile subjects were tested for place preferences to environments associated with 10 mg/kg cocaine, a dose that is a good threshold dose for age-related shifts in sensitivity. A second group of subjects was tested for impulsive choice with a delayed discounting task, with training beginning at 23 days of age.

Results: We found a significant effect of 6-OHDA treatment in both place preferences for cocaine and impulsivity that interacted with sex. 6-OHDA-treated males had a greater preference for the cocaine-associated environment than vehicle-treated males, whereas 6-OHDA lesions reduced cocaine preferences in females (Condition X Sex interaction: $F_{1,15} = 6.12$, $p < 0.05$). 6-OHDA treatment also increased impulsive choice in males 2.23-fold relative to controls, but was without effect in treated females (Condition X Sex interaction: $F_{1,18} = 5.04$, $p < 0.05$). In a subset of subjects, preliminary investigations show a significant correlation ($r = 0.56$) between delayed discounting and cocaine place preference.

Discussion: Our data raise the possibility that reduced cortical levels of dopamine during early development play a significant role in risk for SUD and impulsivity in males, but not females. Whether early identification with measures of delayed discounting can accurately predict later sensitivity to the rewarding effects of substances of abuse may help us identify individuals who could maximally benefit from early interventions. This relationship may only hold for males, and not females. Males are generally considered more at risk for ADHD than females, although this may reflect issues with diagnosis as females are less likely to exhibit externalizing behaviors. Our data in females suggest that other factors other than reduced cortical dopamine play a role in female ADHD symptoms or that the dopamine system shows a different developmental profile in females that may render it less sensitive to manipulation at our selected age.

Disclosure: H. MacGillivray: None. B. Thompson: None. S. Andersen: None.

3. Binge Eating Sucrose Activates Gene Pathways Involved in Substance Abuse

Nicole Avena*, Firas Kobaissy, Miriam Bocarsly, Mark Yang, Bart Hoebel, Mark Gold

University of Florida, Gainesville, FL

Background: Previous studies have shown that binge eating of sucrose produces behavioral and neurochemical changes that are similar, albeit smaller in magnitude, to the effects of some drugs of abuse. These behaviors include escalation of sucrose intake in the first hour, opiate-like withdrawal, and signs of craving. Concomitant brain changes include the repeated release of dopamine (DA) in the nucleus accumbens (NAc) with each binge, and alterations in the expression and binding of DA and opioid receptors. The present study used gene array analysis to search for gene pathways that are activated by binge eating of sucrose and during sucrose abstinence. The hypothesis was that similar gene pathway profiles may emerge as seen during dependency and withdrawal from some drugs of abuse.

Methods: Male Sprague-Dawley rats were maintained on either (1) *ad libitum* standard rodent chow and a 10% (w/v) sucrose solution or (2) binge (12-h) daily access to rodent chow and then 10% sucrose solution. In the binge group, sucrose and chow access began 4 h into the dark period. Groups of rats were sacrificed at four different time points: after 1, 2, and 3 weeks of diet access, or after 3 weeks of access followed by 1 month of abstinence from sucrose ($n=2-4/\text{group}$). Affymetrix Rat Genome 230 2.0 arrays (31,099 Probe Sets) were used to measure apparent gene expression in the NAc. For the analysis, each gene probe was considered as differentially expressed only when profile differences were noted at all four time points. SAS PROC MIXED was used for data analysis.

Results: Different profiles between the binge-and *ad libitum*-fed rats were shown for 270 genes based on 0.05 false discovery rate, i.e., approximately 95% of these genes are expected to truly be differentially expressed as a result of the feeding schedules. Gene ontology analysis revealed group-dependent alterations in leptin, opioid receptor, neuropeptide Y, DA type-2 receptor, and serotonin type-2 receptor signaling pathways. Cluster analysis revealed three main profiles, including between-group differences in the sets of genes that were either (1) downregulated after 1 week of sucrose access, (2) downregulated after 3 weeks of sucrose access and then upregulated following the abstinence period, and (3) upregulated after 3 weeks of sucrose access and then downregulated following the abstinence period.

Discussion: Taken together, these data offer insight regarding possible neural mechanisms underlying the behavioral signs of addiction seen with this model of binge eating of sucrose. Further work is necessary to confirm and extend these findings and possibly identify logical targets for neuronal intervention.

Disclosure: N. Avena: None. F. Kobaissy: None. M. Bocarsly: None. M. Yang: None. B. Hoebel: None. M. Gold: None.

4. Blocking Astrocytic Glutamate Uptake in the Prefrontal Cortex, Alone, Does Not Replicate the Depression-Like Effects of Widespread Blockade

Anita Bechtholt-Gompf*, Karen Smith, Catherine John, William Carlezon Jr., Bruce Cohen, Dost Öngür

Harvard Medical School - McLean Hospital, Belmont, MA

Background: Mood disorders are associated with regional brain abnormalities including reductions in glial cell and neuron number, altered glutamatergic neurotransmission, and differential patterns of brain activation. Because astrocytes are modulators of neuronal activity and are important in trafficking the excitatory neurotransmitter glutamate, it is possible that all these pathologies are interrelated and mutually contribute to some of the behavioral signs that characterize depressive disorders. We have previously reported that central (ICV) blockade of astrocytic glutamate uptake produces anhedonia and cognitive impairments - common symptoms of depression - and induces c-Fos expression in areas implicated in motivation and emotion, including the prefrontal cortex (pfc). Because the pfc is affected in mood disorders and is activated after central blockade of astrocytic glutamate uptake, we set out to determine whether microinjections of an astrocytic glutamate uptake blocker into the pfc are sufficient to induce depressive-like signs.

Methods: We microinjected the astrocytic glutamate transporter (GLT-1) inhibitor dihydrokainic acid (DHK), into the pfc and examined the effects in behavioral tests that quantify aspects of mood, including reward and euthymia/dysthymia, by the use of intracranial self-stimulation (ICSS) and place conditioning, respectively.

Results: Intra-pfc DHK at some doses produced modest increases in the minimum frequency that would maintain ICSS, a depressive-like effect that could reflect reduced sensitivity to reward (anhedonia) or aversion (dysphoria). However, higher doses of DHK eliminated ICSS, making interpretation difficult. To help clarify these findings, rats were subjected to place conditioning using DHK as the unconditioned stimulus. DHK in the pfc increased activity during the early conditioning trials and did not establish conditioned place aversions, as reflected by a lack of avoidance of DHK-associated environments during the drug free test.

Discussion: The dose response function for intra-pfc DHK shows modestly diminished reward followed by abrupt and complete cessation of responding in ICSS, suggesting an all-or-nothing dose-response relationship. Because DHK increased activity during conditioning, it is unlikely that the effects observed in the ICSS paradigm were the result of sedation or motor impairment. Since DHK treatment did not induce conditioned place aversions, these data also suggest that intra-pfc DHK does not induce dysphoria. Taken together these data remain ambiguous regarding whether blocking astrocytic glutamate uptake in the pfc induces anhedonia, but future studies examining effects on other reward processes (e.g. cocaine-induced conditioned place preference) may help to clarify this issue.

Disclosure: A. Bechtholt-Gompf: None. K. Smith: None. C. John: None. W. Carlezon: Part 1; serves as a consultant for Lantheus, Inc. and Transcept, Inc. Nothing else to disclose. B. Cohen: None. D. Öngür: Part 4; received drug for a study from Sanofi-Aventis.

5. Altered Hippocampal-Accumbens Synaptic Plasticity in a Developmental Animal Model of Schizophrenia

Pauline Belujon*, Mary Patton, Anthony Grace

University of Pittsburgh, Pittsburgh, PA

Background: Convergent glutamatergic inputs from limbic and cortical structures, such as the ventral subiculum (vSub) of the hippocampus and the prefrontal cortex (PFC), to the nucleus accumbens (NAc) play a unique role in cognitive functions. For example, the vSub is involved in context-dependent processing, and

the PFC in working memory processes. Moreover, neurons from the NAc receive dopaminergic inputs from the ventral tegmental area that play a critical role in modulating their response to both inputs. Functional disruption in these systems is proposed to underlie psychiatric disorders such as schizophrenia. Indeed, in schizophrenia patients, a deficit of prepulse inhibition of the startle response is observed and is proposed to involve gating within the vSub. Moreover, schizophrenia patients show working memory deficits known to involve the PFC. Thus, disruption of the balance between the ventral subiculum and the prefrontal cortex is likely a pathophysiological factor in schizophrenia. In schizophrenia patients, hyperactivity of the hippocampus and reduced mPFC activation are observed during the performance of working memory tasks. Hyperactivity in the hippocampus is thought to cause an imbalance between afferents to the NAc, as well as a dopaminergic hyperfunction. Furthermore, the mPFC exerts a regulatory influence over subcortical structures and a reduced GABA interneuron marker in the PFC is thought to induce unregulated activity in this structure. We have previously shown in normal rats that the mPFC is required for the activation of NAc neurons by the vSub; an influence that is lost when the vSub-NAc pathway is potentiated. The aim of the present study is to examine the response of NAc neurons to vSub activation in an animal model of schizophrenia and how this is modulated by mPFC.

Methods: We used the methylazoxymethanol acetate (MAM) developmental animal model of schizophrenia. *In vivo* extracellular single unit recordings from NAc neurons were performed in anesthetized adult offspring rats of MAM- and saline-treated dams. We examined the response of NAc neurons to single pulse stimulation of the vSub-NAc pathway, and how this is affected by inactivation of the PFC. In addition, high-frequency stimulation, as well as low frequency stimulation, was applied to the vSub-NAc pathway of MAM- and saline-treated rats.

Results: In contrast to control rats, inactivation of the PFC by the sodium channel blocker tetrodotoxin increases the ability of the vSub to drive spike firing in the NAc in MAM-treated rats. Moreover, tetanic stimulation of hippocampal afferents, which induces long-term potentiation (LTP) of the vSub-NAc pathway in normal rats, attenuates the hippocampal drive of NAc neurons. However, low-frequency stimulation of hippocampal afferents, which induces long-term depression (LTD) of the vSub-NAc pathway in saline-treated rats, induces an increase of the hippocampus drive of NAc neurons (LTP) in half of the rats tested.

Discussion: This demonstrates altered synaptic plasticity of the vSub-NAc pathway in MAM-treated rats. Considering the critical role of the mPFC in the modulation of this pathway in normal animals, it is likely that alterations in mPFC and hippocampal inputs selectivity in the MAM-treated rat may lead to dysregulation of synaptic plasticity in subcortical structures.

Disclosure: P. Belujon: None. M. Patton: None. A. Grace: None.

6. Witnessing Physical Stress Induces an Anxiety- and Depression-Like State in Adult Mice

Brandon Warren, Sergio Iniguez, Eric Nestler, Carlos Bolanos-Guzman*

Florida State University, Tallahassee, FL

Background: It is well known that exposure to severe stress increases the risk for developing mood disorders. While much has been learned from animal models of traumatic stress, current paradigms emphasize physical stressors, while models of emotional stress focus on parental neglect and social isolation stressors. However, it is common for post-traumatic stress disorder to develop in individuals who simply witness intense violence. Therefore, it is critical to develop animal models that will allow for independent assessment of the neurobiological consequences of emotional stress (ES). Here we introduce a novel social stressor that is insulated from the effects of physical stress.

Methods: In this study, male C57/BL6J mice were forced to witness the social defeat of another mouse. Briefly, the home cage of a male CD-1 retired breeder mouse was divided by a Plexiglas divider into two adjacent compartments. An adult male C57/BL6J mouse was introduced into the compartment territorialized by the CD-1 mouse where it was repeatedly attacked and demonstrated escape-like behaviors, vocalizations, and submissive posturing, while a second male C57/Bl6J mouse witnessed this interaction from the adjacent compartment (ES).

Results: Here we demonstrate that 10 days of ES induces long-lasting deficits in a battery of behavioral assays designed to assess changes in mood. Specifically, mice exposed to ES show a robust social avoidance 24 hours after the last exposure to ES. ES also increases anxiety- and depression-like behaviors as measured by the elevated plus maze, elevated O-maze, the open field, the forced swim, and sucrose preference tests. Changes in serum corticosterone levels accompanied these behavioral deficits. Moreover, we observed altered gene expression within the ventral tegmental area (VTA), an area highly implicated in both responses to stress and the etiology of mood disorders. Namely, we observed increased expression of GSK3 β , ERK2, and the HCN family of cation channels.

Discussion: Taken together, these data indicate that witnessing traumatic stress is a potent stressor in mice capable of inducing long-lasting neurobiological alterations.

Disclosure: B. Warren: None. S. Iniguez: None. E. Nestler: None. C. Bolanos-Guzman: None.

7. Elevated Levels of the Endogenous Alpha7 Antagonist Kynurenic Acid Dysregulate Cortical Glutamatergic and Cholinergic Transmission and Impair Cognitive Flexibility: Relevance to Schizophrenia

Kathleen Alexander, Hui-Qiu Wu, Julie Brooks, Robert Schwarcz, John Bruno*

The Ohio State University, Columbus, OH

Background: Kynurenic acid (KYNA), an astrocyte-derived, endogenous antagonist of the alpha7 nicotinic acetylcholine receptor (alpha7nAChR), is elevated in the prefrontal cortex (PFC) of persons with schizophrenia (SZ). This elevation may contribute to the development of dysregulated cortical transmission and cognitive deficits. In developing a KYNA-based animal model of SZ, we manipulated brain KYNA levels in intact rats and measured levels of glutamate and ACh - two transmitters central to the mediation of cognitive behavior - in the PFC. We also determined the effects of elevated KYNA levels on performance of a perceptual set-shifting task (a task that reveals deficits in SZ) and explored if performance relies upon cholinergic and glutamatergic transmission in the PFC.

Methods: Studies were performed using male Wistar rats. First, KYNA levels in the PFC were elevated following the systemic application of kynureanine (kyn; 25, 100 mg/kg, i.p.), the bioprecursor of KYNA. Extracellular levels of KYNA and glutamate were determined using both microdialysis/HPLC methods as well as a high resolution *in situ* glutamate microelectrode. Second, KYNA levels were modified locally following intra-PFC perfusions of kyn (2.5 μ M, to increase levels) or S-ESBA, a selective inhibitor of KYNA neosynthesis (5 mM, to reduce levels). Third, the effects of local KYNA (100 nM) administration on amphetamine (2.0 mg/kg)-stimulated ACh release in PFC were determined. Fourth, the necessity of prefrontal cholinergic and glutamatergic transmission for performance of a perceptual set-shifting task was studied. Discrimination was based on 3 perceptual elements, i.e. texture of the pot, digging material within the pot, and odor of the digging material. Following acquisition of the discrimination, animals were tested for several reversals, an intra-dimensional shift (IDS) and an extra-dimensional shift (EDS). The effect of systemic kyn (100 mg/kg, i.p.) on task performance was examined with and without the alpha7nAChR agonist galantamine (co-administered at 3.0 mg/kg, i.p.). Finally, in order to more selectively link glutamate and ACh to task performance, rats were also tested following bilateral

intra-PFC infusions of nicotinic (mecamylamine, MEC; 10 µg/side), muscarinic (scopolamine, SCOP; 8 µg/side), or NMDA (MK-801; 3 µg/side) receptor antagonists.

Results: Systemic administration of kyn caused dose-dependent, reversible increases in extracellular KYNA levels in the PFC (max increase 20-fold above baseline, 3 hr following injection). This was accompanied by decreases in basal extracellular levels of glutamate. Similar effects on prefrontal KYNA and glutamate, respectively, were obtained by local perfusion of kyn. The kyn-induced reductions in glutamate were secondary to elevated KYNA levels, as they were prevented by co-perfusion of kyn with S-ESBA. Moreover, local perfusion of KYNA blocked the ability of amphetamine to stimulate prefrontal ACh. Behaviorally, systemic administration of kyn produced performance deficits in the set-shifting task. Compared to saline (i.p.)-treated controls (N = 5), rats treated with kyn (N = 4) showed no effect on initial acquisition [9 (drug) vs. 7 (saline) trials] or IDS (6 vs. 6 trials), yet were significantly impaired in the first reversal (21 vs. 12 trials) and the EDS (18 vs. 8 trials). Notably, co-administration of the α_7 nAChR agonist galantamine (N = 4) eliminated the kyn-induced deficits in first reversal [13 (kyn/galant) vs. 12 (saline) trials] and EDS (7 vs. 8 trials). Selective reductions in prefrontal glutamatergic and cholinergic transmission also resulted in marked impairments in the set-shifting task. Thus, compared to rats receiving intra-PFC infusions of aCSF (N = 4), rats treated with MEC (N = 7), SCOP (N = 6), and MK-801 (N = 3) exhibited elevated mean trials to criterion in first reversal [aCSF, 10; MEC, 20; SCOP, 25; MK-801, 16 trials] and EDS [aCSF, 9; MEC, 13; SCOP, 12; MK-801, 17 trials].

Discussion: Jointly, these results support the idea that elevated KYNA levels, by inhibiting alpha γ nAChRs, dysregulate prefrontal glutamatergic and cholinergic transmission and impair perceptual set-shifting, particularly at the level of reversals and EDS. These effects reproduce impairments seen in individuals with SZ. This duplication of neurochemical and specific cognitive deficits suggests that acute increases in KYNA in rats may constitute a new validated animal model of SZ. Our results also imply that compounds designed to normalize KYNA levels in the brain (e.g. inhibitors of KYNA biosynthesis) can be used as investigational tools in SZ research and may hold promise for the treatment of SZ.

Disclosure: K. Alexander: None. H. Wu: None. J. Brooks: None. R. Schwarcz: Part 4; Mitsubishi-Tanabe Pharma (Japan). J. Bruno: None.

8. Development of a High-Throughput Screen In *C. elegans* to Identify New Treatments for Schizophrenia

Xin Wang, David Linden, Bruce Cohen, Edgar Buttner*

McLean Hospital, Belmont, MA

Background: Treatment of schizophrenia has been hindered by the limited efficacy of many currently available antipsychotic medications and the toxic side effects of these drugs. The molecular mechanisms underlying the biological effects of antipsychotic drugs remain unclear. In previous pharmacogenomic studies in *C. elegans*, we found that clozapine, the most effective medication for the treatment of schizophrenia, arrests *C. elegans* development. Using a genome-wide RNAi screen and a candidate gene screen for suppressors of clozapine-induced larval arrest in *C. elegans*, we identified insulin/IGF-1 signaling (IIS) as a candidate signal transduction pathway through which clozapine may produce its therapeutic and/or toxic effects in humans. Subsequently, Weeks *et al* showed that other antipsychotics also activate IIS in *C. elegans*. Our preliminary data also shows that AKT, a key component of IIS, is activated by clozapine in human neuroblastoma SH-SY5Y cells.

Methods: To find small molecules suitable for the treatment of schizophrenia, we are developing a chemical biological high-throughput screen (HTS) for compounds that phenocopy the biological effects of antipsychotics in *C. elegans*. The primary screen will identify compounds that cause developmental arrest or delay in *C. elegans*. The secondary screen will identify positives from the primary screen that

produce developmental arrest suppressible by *age-1(lf)*. Our primary screen will yield some nonspecific positives that arrest *C. elegans* development but that are not antipsychotic-like drugs. The secondary screen is designed to eliminate these compounds.

Results: We have shown that *daf-2(lf)* and *age-1(lf)* mutants suppress clozapine-induced larval arrest. Developmental delay suppressible by *age-1(lf)* may be a phenotype specific to antipsychotic drugs, since we have found that other examples of drug-induced larval arrest, such as ivermectin-induced larval arrest, are not suppressible by *age-1(lf)*. The primary screen will utilize an adult-specific *vit-2::GFP* reporter. Since only gravid adults will exhibit fluorescence in the HTS, wells containing arrested animals can be identified by a lack of fluorescence. Using *age-1(hx546); vit-2::GFP* animals, we will then conduct a secondary screen for wells containing GFP-positive adults, i.e. animals that have escaped drug-induced larval arrest.

Discussion: IIS is well-conserved across species, including humans, and plays an important role in development, signal transduction, and synaptogenesis in the mammalian nervous system. Recent studies have underscored the potential importance of this pathway in the pathophysiology of schizophrenia. These findings, and our studies of clozapine's effects on IIS, suggest that IIS is involved in the expression of symptoms in schizophrenia and the therapeutic effects of antipsychotic drugs.

Disclosure: X. Wang: None. D. Linden: None. B. Cohen: None. E. Buttner: None.

9. Enhanced CREB Activity in the Nucleus Accumbens Shell Produces Anhedonia and Impaired Extinction of Conditioned Fear

John Muschamp, Ashlee Van'T Veer, Aram Parsegian, Miranda Gallo, Melissa Chen, Rachel Neve, Edward Meloni, William Carlezon Jr.*

Harvard Medical School, Belmont, MA

Background: Stress is thought confer vulnerability to psychiatric illnesses like major depression and anxiety disorders. The mechanisms by which stress induces lasting changes in mood and behavior are not fully understood, but the high comorbidity of depression and anxiety suggests some overlap in their pathophysiology. One candidate for a common pathway for these disorders is the activity of the transcription factor CREB (cyclic AMP response element binding protein) in the nucleus accumbens shell (NAS), a brain structure known to be involved in encoding rewarding and aversive states. Previous work shows that forced swim stress activates CREB in the NAS and that viral vector-induced elevation of NAS CREB expression produces depressive-like effects in various behavioral models. This effect is due in turn to the induction of dynorphin by CREB and can be mimicked by the administration of agonists at kappa opioid receptors (KOR).

Methods: The level of activated (phosphorylated) CREB (pCREB) in the NAS of rats subjected to footshock stress was measured by western blot. To manipulate levels of CREB expression, viral vectors encoding CREB or a dominant negative form of the protein (mCREB) were injected into the NAS and data from these animals compared to that of control animals that received injections of vehicle or vectors encoding a reporter gene (LacZ). In one set of experiments, injections were made after measuring baseline reward thresholds using a rate-frequency procedure for intracranial self stimulation (ICSS). The effect of virus injections on reward thresholds was then assessed daily for 8 days. In parallel studies, injections were made either before or after fear conditioning in which a light stimulus and footshock were paired. Subsequently, the ability of the light stimulus to potentiate the acoustic startle reflex was measured both before and after extinction training sessions that consisted of repeated representation of the light. In a final set of experiments, the same behavioral paradigms were used to assess the ability of the KOR agonist U50,488 to influence reward thresholds, or to affect the expression or extinction of conditioned fear by giving acute microinjections of drug or vehicle into NAS.

Results: Consistent with previous findings using other stressors, footshock stress elevated pCREB levels in NAS. Overexpression of CREB in NAS elevated ICSS thresholds, suggesting anhedonia, while mCREB produced opposite effects. Although the elevation or reduction of CREB in NAS had no effect on the acquisition of conditioned fear, elevated CREB markedly disrupted extinction of conditioned fear. Although the pro-depressive effects of elevated CREB on ICSS thresholds were mimicked by intra-NAS injection of U50,488, this drug had no effect on levels of fear potentiated startle when given before fear conditioning or before extinction training.

Discussion: These findings demonstrate that activation of CREB in the NAS produces multiple behavioral signs (anhedonia, impaired extinction) characteristic of experience-dependent psychiatric conditions such as post-traumatic stress disorder (PTSD). Although CREB activation is a common trigger, expression of these individual signs appears to involve divergent downstream mechanisms, as stimulation of KORs produces anhedonia but not resistance to extinction.

Disclosure: J. Muschamp: None. A. Van't Veer: None. A. Parsegian: None. M. Gallo: None. M. Chen: None. R. Neve: None. E. Meloni: None. W. Carlezon Jr.: Part 3; Lantheus, Inc., Transcept, Inc.

10. Prefrontal Cortical and Striatal Gene Expression Changes in the NVHL Neurodevelopmental Model of Schizophrenia With and Without a Co-Occurring History of Cocaine Sensitization

Robert Chambers*, Jeanette McClintick, Alena Sentir, Sarah Berg, Kwang Ho Choi, Howard Edenberg

Indiana University School of Medicine, Indianapolis, IN

Background: Neonatal ventral hippocampal lesions (NVHLs) in rats produce multiple behavioral, neurocircuit, and developmental aspects of schizophrenia, including addiction vulnerability. Previous work suggests high rates of substance disorders and addiction vulnerability in mental illness (i.e. dual diagnosis) results from an interaction of pathologies underlying mental illness and addiction within prefrontal cortical/striatal circuits. This study examined how gene expression changes occur within these circuits as a consequence of the independent and co-occurring effects of NVHLs and behavioral sensitization to cocaine.

Methods: We prepared groups of NVHL vs. SHAM-operated rats that underwent cocaine sensitization (15 mg/kg i.p. cocaine daily x 5 days) vs. serial saline injections in early adulthood. Lesion status x drug history groups were rigorously counterbalanced by maternal litters of origin. Two weeks after the injection series, rats were sacrificed and brains dissected for rapid recovery of tissue blocks from the medial prefrontal cortex (MPFC), nucleus accumbens (NAC), and Caudate-Putamen (CAPU). These samples were then analyzed with Affymetrix (Rat Gene 1.0 ST) gene expression arrays containing 27,000 mRNA probe sets.

Results: Significant differences in behavioral sensitization to cocaine emerged involving lesion and drug history main effects and interactions in which NVHL-COC rats showed the most locomotor activation and SHAM-SAL rats the least by day 5. A quality control procedure using a pre-set expression level cut-off retained approximately 24,000 probesets for each region. Patterns of gene expression were identified as differences in the identifications and number of genes significantly impacted by lesion status, drug history and their interaction on the $p < 0.01$ and $p < 0.001$ levels within and across the 3 brain regions. Expression patterns generally varied according to several major themes: 1) NVHLs impacted a greater number of genes than drug history across all three brain regions; 2) NVHLs and drug history predominantly impacted the largest numbers of genes in the NAC compared to MPFC and CAPU; 3) NVHLs predominantly caused down regulation of gene expression in the MPFC and NAC, but up regulation in the CAPU; 4) cocaine history predominantly caused up regulation in all brain regions, but most robustly sub-cortically; 5) lesion and drug history effects preferentially impacted genes that

independently also show the most variation in expression based on non-specific genetic and environmental conditions (mom/litter of origin as an independent effect); and 6) substantial proportions (>50%) of genes impacted at the $p < 0.001$ level by NVHLs or drug history within the CAPU were regulated as a significant positive correlation with degree of behavioral sensitization across treatment groups.

Discussion: This data set holds considerable potential for understanding the complex interactions of mental illness and addiction pathologies as an integrated disease process that involves multiple brain regions and changes in the expression patterns of hundreds of individual genes. The ability to select genes of interest based on the effects of lesion status, drug history and/or correlations between expression levels and behavioral phenotype provides multiple avenues for identifying expression changes most critically involved in the cellular functions and cortical-striatal network aberrations that underpin addiction vulnerability in mental illness.

Disclosure: R. Chambers: None. J. McClintick: None. A. Sentir: None. S. Berg: None. K. Choi: None. H. Edenberg: None.

11. In Vivo Suppression of Dopamine D₃ Receptor Expression in Adult Rats Using Lentiviral Vectors

Wei-Li Chang*, Xianjin Zhou, Katie Schaukowitch, Michelle R. Breier, Richard L. Saint Marie, Neal Swerdlow

University of California San Diego, La Jolla, CA

Background: Virtually all approved medications for schizophrenia act at dopamine (DA) receptors, antagonizing the D₂ and D₃ DA receptor subtypes in a nonselective manner. D₃-selective compounds may represent a novel class of antipsychotics that lack the major side effects of non-selective antagonists. Prepulse inhibition (PPI) of the acoustic startle reflex, an operational measure of sensorimotor gating, can be modified by DA-active drugs in humans and rodents. This behavioral test is used in rodents as a preclinical model to predict antipsychotic potential. Unfortunately, many drugs that act on D₃ receptors do not affect PPI in mice, and thus traditional "knockdown" strategies cannot be used to assess the *in vivo* receptor subtype specificity of drugs that display preferential binding to D₃ receptors *in vitro*. This study aims to use viral vectors to suppress D₃ receptor expression in the adult rat brain for the purpose of testing receptor specificity of DA-active drugs in awake behaving animals.

Methods: A pseudotyped lentiviral vector packaging system is used to deliver one of three plasmids containing sequences for small-hairpin RNAs (shRNA) that have been shown to successfully silence D₃R expression in the rat brain or a CMV-EGFP expression cassette (vehicle condition). Virus containing one of 4 different constructs is infused at a high titer into the nucleus accumbens (NAC) bilaterally (1 μ l/side at 0.2 μ l/min). Adult male Sprague-Dawley rats are tested for PPI prior to injection surgery and again on the day of tissue collection. Successful infection by lentivirus vector is assessed by Western blot or immunohistochemical staining for GFP in brain tissue collected from vehicle group. D₃ receptor expression is measured at two-week intervals 2 to 10 weeks after lentivirus infusion using either Western blot or rt-PCR.

Results: Two weeks after lentiviral infusion, GFP expression can be seen in the NAC with Western blot. Immunohistochemical staining of GFP demonstrated the anatomical extent of lentiviral uptake in the region of the accumbens. At this time point, expected patterns of startle modulation were detected across groups, including significant reflex habituation ($p < 0.0001$) and intensity-dependent prepulse inhibition ($p < 0.0001$). Levels of startle magnitude, habituation and PPI did not differ significantly across the 4 viral constructs, though post-injection startle magnitude among rats infused with D₃ shRNA tended to be elevated compared to lentivirus control rats ($d = 1.36$), suggesting that the active D₃ shRNA infusions were bioactive. rt-PCR and Western blot data for D₃ receptor expression weeks 2-10 post injection will be presented, along with complete behavioral data from pre- and post-infusion test days.

Discussion: Lentiviral injections into the nucleus accumbens yielded anatomically localized uptake and expression of vector-delivered genetic material. Preliminary behavioral data suggest that this material did not interfere with basal startle modulation, thus clearing the way for future pharmacological probes of D₃ function in these rats. The present findings suggest that lentiviral vector-mediated suppression of D₃ receptor expression in brain regions relevant to sensorimotor gating may be a viable method of assessing the effects of selective D₃ receptor antagonism in a predictive model for antipsychotic potency. Supported by MH087109 and MH068366 and AG000216.

Disclosure: W. Chang: Part 4; MH087109, AG000216. X. Zhou: None. K. Schaukowitch: None. M. Breier: None. R. Saint Marie: None. N. Swerdlow: Part 1; Allergan. Part 4; MH068366.

12. D-Serine Produces Ketamine-Like Effects in Animal Models of Depression

Tyson Tragon, Daniel Austin, Gregory Rompala, Anahita Hamidi, Kazu Nakazawa, Guang Chen*

NIMH-NIH, Bethesda, MD

Background: Glial dysfunction has been implicated by human and animal studies as a part of the underlying neurobiology of depression. Glutamate evokes releases of D-serine, from astrocytes. D-serine, in turn, stimulates NMDA NR1 receptor. We hypothesize that D-serine, an astrocyte gliotransmitter, modulates behaviors related to depression. **Methods:** The behavioral effects of acute D-serine administration were examined in the forced swim test (FST), learned helplessness paradigm (LH), and serotonin depletion paradigm (SD) with sexual pleasure-seeking activity as the respective outcome measures.

Results: D-serine administration resulted in significant immobility reductions in the forced swim test (50-70%, t-test, p < 0.05) without affecting locomotion in the open field test. The administration also significantly improved helplessness, as measured by the escape latency (Two-way repeated measure ANOVA, F(1, 406) = 5.812, p = 0.0302), number of escapes (t(14) = 2.177, p = 0.0235), and percentage of helplessness animals (Chi-square = 4.267, p = 0.0389). Serotonin depletion caused significant reduction (t-test, p < 0.05) in sexual pleasure-seeking activities in male rodents monitored with the female urine sniffing test. D-serine administration abolished the reduction.

Discussion: Previous data showed that ketamine treatment influences measures related to depression in the FST, LH and SD. Present data demonstrated that D-serine produced similar effects in these paradigms. These data imply that D-serine may produce ketamine-like effect in depression and support glial dysfunction as a part of underlying mechanism of depression. The data also support targeting NMDA NR1 receptor to develop novel antidepressant.

Disclosure: T. Tragon: None. D. Austin: None. G. Rompala: None. A. Hamidi: None. K. Nakazawa: None. G. Chen: None.

13. Altered NMDA Receptor Signaling Affects the Extinction of Drug-Associated Environmental Cues as Measured by a Novel Variation of the Psychostimulant Sensitization Paradigm

Joseph Coyle*, Michael Benneyworth

Harvard Medical School, Belmont, MA

Background: The development of more effective therapeutic interventions for both schizophrenia and addiction can be advanced by understanding the co-morbidity of these diseases and by delineating the overlapping neurobiology that predisposes schizophrenic patients to such high rates of substance abuse. Considerable evidence supports the hypothesis that schizophrenia results from hypofunctional NMDA receptor signaling, and recent findings point towards NMDA receptor-mediated mechanisms controlling the neuroplastic changes responsible for addiction. In addition to the agonist glutamate, NMDA receptor activation requires the binding of a co-agonist to the glycine modulatory site (GMS). D-serine is the primary GMS agonist in the

forebrain. We used a genetically-modified mouse strain carrying a homozygous null mutation (-/-) in serine racemase (SR), a mutant model with etiological relevance to schizophrenia, to develop a phenotype for susceptibility to addiction. The null mutation results in nearly absent central nervous system D-serine levels and diminished NMDA receptor activity [1].

Methods: The present studies investigated the outcome of altering the availability of D-serine on the experimental measures of amphetamine (AMPH) sensitization and AMPH-induced, context-dependent hyperactivity. AMPH sensitization involved 5 daily injections of AMPH (3.0 mg/kg, s.c.) or vehicle. Activity was measured for 60 min immediately following treatment. Sensitization was assessed in a test session after 14-day drug-free period. For the sensitization test all animals were treated with AMPH (3.0 mg/kg). Contextual hyperactivity was assessed in 60 min sessions beginning 3 days after the sensitization test, and the subsequent extinction was observed for a total of 10 sessions.

Results: <br WT mice subjected to a sensitizing regimen of AMPH respond at near maximal levels of activity within 15 minutes (compared to maximum activity reached at 35 min in control subjects). This dramatic leftward shift in the time course of activity suggests an anticipatory response based upon the experimental (context) cues. This was further demonstrated by observing drug-free activity, during which AMPH-treated mice displayed a 70% increase in activity as compared to vehicle-treated controls when placed in the test chamber. This contextual hyperactivity was extinguished with repeated exposure to the test chamber in the absence of AMPH treatment. Further experiments were done to demonstrate the context-dependency of the hyperactivity and the extinction session-dependency of the loss of that hyperactivity. SR-/- mice were compared to WT littermates for the induction of AMPH sensitization and the extinction of contextual hyperactivity. Acquisition of sensitization was somewhat slower in the SR-/- mice. After a 14-day drug withdrawal, SR-/- mice displayed reduced AMPH sensitization as compared to WT littermates. SR-/- treated with AMPH showed less contextual hyperactivity than WT littermates, but the contextual hyperactivity of the SR-/- was significantly more persistent than that in WT littermates, failing to extinguish to the same degree.

Discussion: <br The psychostimulant sensitization paradigm itself is a widely used model of the long-term behavioral and molecular changes caused by drugs of abuse. However, this contextual hyperactivity and extinction represent a novel way to investigate the behavioral and molecular effects of repeated drug administration that are not dependent upon the presence of the drug. The phenotype of the SR mutant mouse is consistent with previous reports that NMDA receptor activity is important for the expression of psychostimulant sensitization [2, 3]. The demonstration of the altered extinction of the contextual hyperactivity is suggestive of a behavioral phenotype that relates NMDA receptor hypofunction to increased susceptibility to addiction-related behavior after long-term drug intake 1. Basu, A.C., et al., Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Mol Psychiatry, 2009. 14(7): p. 719-27. 2. Mao, L.M., et al., Stability of surface NMDA receptors controls synaptic and behavioral adaptations to amphetamine. Nat Neurosci, 2009. 12(5): p. 602-10. 3. Zweifel, L.S., et al., Role of NMDA receptors in dopamine neurons for plasticity and addictive behaviors. Neuron, 2008. 59(3): p. 486-96.

Disclosure: J. Coyle: Part 1; Abbott Pharm: consultant, stock; Puretech; BristolMeyerSquibb; Lilly. Part 2; Biovail. Part 4; BristolMeyerSquibb. M. Benneyworth: None.

14. Effects of Prazosin, an α_1 -Adrenergic Receptor Antagonist, on the Seeking and Intake of Ethanol and Sucrose in Alcohol-Preferring (P) Rats

Cristine Czachowski*, Terril Verplaetse, Dennis Rasmussen, Janice Froehlich

Indiana University School of Medicine, Indianapolis, IN

Background: Prazosin, an α_1 -adrenergic receptor antagonist, has previously been shown to decrease relapse alcohol drinking in

treatment-seeking, alcohol-dependent men (Simpson et al., 2009). Prazosin has also been shown to block alcohol dependence-induced increases in operant responding for alcohol by Wistar rats (Walker et al., 2008) and to decrease voluntary alcohol drinking in rodents selectively bred for alcohol preference (alcohol-preferring or "P" rats) (Rasmussen et al., 2009). This study extended these findings by using a paradigm that allows for separate assessment of whether a drug alters motivation to seek versus to consume a reinforcer, such as alcohol, and whether the effect of a drug is specific for a given reinforcer, such as alcohol.

Methods: 15 male P rats were acclimated and trained for 8 weeks to complete an operant response requirement that resulted in access to either 2% sucrose (sucrose group) or 10% alcohol (alcohol group) for 20 minutes a day. A 4-week consummatory testing phase consisted of the animals bar-pressing to essentially "pay" a specified amount up front to gain access to unlimited alcohol (or sucrose) for a 20-minute period. A 4-week appetitive testing phase examined how much the animals would bar-press or "pay" up front during an extinction session when no reinforcer could be obtained - hence measuring the seeking or motivation to obtain alcohol or sucrose, with no limit placed on the number of responses that could be made. On consummatory test days and on appetitive test days, rats were given IP injections of either vehicle or one of three doses of prazosin (0.5, 1.0, or 1.5 mg/kg; balanced design) at 30 min prior to test. On consummatory test days, lick latency and total fluid intake were recorded. On appetitive test days (no reinforcer available, "extinction") bar-press latency and total number of bar-press responses were recorded.

Results: Vehicle-treated rats that were bar-pressing for alcohol averaged 69.7 responses and 0.90 g/kg of alcohol consumed. Vehicle-treated rats that were bar-pressing for sucrose averaged 104.7 responses and 1.10 g/kg sucrose consumed. When compared to vehicle, prazosin decreased bar-pressing for both alcohol ($p < 0.05$) and sucrose ($p < 0.05$) in a dose-dependent manner. With regard to consumption, the highest dose of prazosin (1.5 mg/kg) decreased intake of both alcohol and sucrose ($p < 0.001$). When compared to vehicle, prazosin had a stronger suppressive effect on alcohol seeking and drinking than it did on sucrose seeking and drinking. Prazosin (1.5 mg/kg BW) decreased alcohol-seeking and alcohol drinking by an average of 76% and 67%, respectively, but decreased sucrose-seeking and sucrose-drinking by an average of only 44% and 39%, respectively. Interestingly, prazosin (1.5 mg/kg) increased the latency to bar press and to lick for alcohol but not for sucrose ($p < 0.05$). This suggests that prazosin may decrease the motivation to initiate drinking of alcohol. The fact that latencies to bar press or lick for sucrose were not altered by prazosin indicates that the decreased latencies for alcohol were not due to prazosin-induced motor-impairment or malaise.

Discussion: Prazosin significantly decreased both the seeking and the drinking of alcohol and sucrose reinforcers, with a stronger effect on alcohol than on sucrose. Prazosin also increased the latency to initiate alcohol seeking and drinking, and decreased alcohol seeking at lower doses than those required to decrease alcohol drinking. These findings extend previous preclinical and clinical findings and suggest that prazosin decreases motivation to initiate alcohol drinking. Prazosin may be effective in decreasing both motivation to drink and amount of alcohol consumed by patients seeking treatment for alcohol use and abuse. RO1 AA018604 (JCF and DDR); P60 AA007611 (JCF and CC); P20 AA017839 and Dept of Veterans Affairs (DDR).

Disclosure: C. Czachowski: None. T. Verplaetse: None. D. Rasmussen: None. J. Froehlich: None.

15. Development of a Novel probabilistic Reward Task in Rats That is an Analogue of a Human Reward Task Used to Assess Anhedonia: Implications for Translational Research

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

University of California, San Diego, La Jolla, CA

Background: Mood disorders, including major depression, are characterized by anhedonia, defined as decreased interest in pleasurable or

reduced reactivity to rewarding events. It is believed that anhedonia results from abnormal processing of rewarding stimuli. Clinical evidence suggests that patients with major depression display dysfunctional processing of positively reinforcing stimuli when assessed using a probabilistic reward task that is based on a differential reinforcement schedule [Pizzagalli et al. (2008) *J Psychiatr Res* 43(1) 76-87]. Briefly, this task involves exposure to two different visual stimuli that are difficult to discriminate, each requiring a different response to lead to reinforcement. Correct responses to one stimulus ("rich" stimulus) are rewarded three times more frequently compared to correct responses to the other stimulus ("lean" stimulus). Healthy human subjects modulate their behavior over the time of testing as a function of prior reinforcement by gradually developing a bias towards increased responding for the "rich" stimulus. In contrast, depressed subjects fail to develop this response bias for the more frequently rewarded "rich" stimulus. The result is a quantitative task that objectively measures deficits in reward processing in anhedonic individuals. The goal of the present study was to develop an analogous probabilistic reward task in rats that can be used to conduct translational preclinical studies. Based on the data from human studies, we hypothesized that healthy rats would develop a similar response bias for the more frequently rewarded "rich" stimulus than the less frequently rewarded "lean" stimulus.

Methods: Two separate groups of male Wistar and Long-Evans rats were trained on the following procedures to investigate potential strain differences. Initially, food restricted rats were trained in operant boxes to press a lever to receive a food pellet as a reward. Rats were then presented with one of two tones varying in length, but identical in all other parameters, and trained to subsequently press one of two levers associated with each tone length. Once these associations were learned, defined as more than 80% accuracy, the difference between the two tone lengths was made more ambiguous during a test session to allow positive reinforcement of correct responses to influence subsequent behavior. One hundred trials were conducted during the test using identical parameters as the probabilistic reward task used in human subjects. Briefly, correct responses on the lever associated with either the short or the long tone (counterbalanced) were reinforced three times more (i.e., "rich" condition) than correct responses on the other lever (i.e., "lean" condition).

Results: Both Wistar and Long Evans rats developed a response bias toward the "rich" stimulus that is comparable to the response bias quantified in healthy human subjects, with results from the Wistars being slightly more equivalent to results from human subjects. That is, rats, similar to healthy human subjects, display a minimal response bias during the initial one-third of the 100 trials presented during a session, when rats and humans are first exposed to the probabilistic nature of the reinforcement schedule. During the final one-third of the session trials, rats and humans display a bias for the response associated with the "rich" stimulus; that is, they respond more on the lever associated with the "rich" stimulus than the lever associated with the "lean" stimulus. Other measures of the probabilistic reward task, such as ability to consistently discriminate between the different tone stimuli throughout the test session and increased accuracy for the "rich" versus "lean" stimuli over the time of testing, were also comparable to results obtained with healthy human subjects.

Discussion: The results indicate that rats alter their response patterns over time as a function of reinforcement history, similar to humans. These data are the first to provide an analogue of the probabilistic reward task in human subjects that can be used to quantify dysfunctional reward processing in anhedonic individuals, and may be used to investigate the neurobiological substrates that underlie reward responsiveness and anhedonia in rodents.

Disclosure: A. Der-Avakian: None. M. D'Souza: None. D. Pizzagalli: Part 1; Advanced Neurotechnology North America, AstraZeneca. Part 4; Advanced Neurotechnology North America. A. Markou: Part 1; Intracellular Therapeutics, Inc., Lundbeck Research USA, Inc., Bristol-Myers Squibb Co., F. Hoffman-La Roche, Inc., Pfizer, AstraZeneca, Abbott GmbH and Co. Part 4; Intracellular Therapeutics,

Inc., Lundbeck Research USA, Inc., Bristol-Myers Squibb Co., F. Hoffman-La Roche, Inc., Pfizer, AstraZeneca.

16. Conditioned Cues that Indicate the Presence or Absence of Ethanol Differentially Activate Areas within the Extended Amygdala in Alcohol-Preferring (P) Rats

Zheng-Ming Ding, Sheketha Hauser, Gerald Deehan, William Truitt, William McBride, Zachary Rodd*

Indiana University: School of Medicine, Indianapolis, IN

Background: Stimuli can readily become associated with the availability (excitation) or unavailability (inhibition) of a reinforcer. The presentation of odor cues previously associated with the availability of alcohol (EtOH) can elicit EtOH-seeking in animal models of drug-seeking. In humans, conditioned cues may elicit drug-seeking when presented in non-drug environments. The current series of studies examined the effects of exposure to various conditioned odor cues on Pavlovian Spontaneous Recovery (PSR; context-induced seeking) as a measure of alcohol-seeking behavior.

Methods: A unique odor was used for each condition tested, but all rats received exposure to all odors (3 total). In all experiments, EtOH-naïve alcohol-preferring P rats were self-trained on a standard two-lever (15% EtOH vs water) operant paradigm using daily 1-hr sessions. Daily EtOH exposure for a 10-week period in the operant chambers was constantly paired with the presence of an odor (CS+). Rats were then exposed to 7 consecutive extinction sessions (no water or EtOH) which was paired with an additional odor (CS-). From day 58-77 (overlapping both EtOH self-administration and extinction) rats were exposed to another odor in a novel environment (CS neutral or CS0). After extinction training, rats were maintained in their home cages for two weeks. In the first experiment, rats were randomly assigned to 4 groups (no odor, CS+, CS-, or CS0) which received the corresponding odor for 4 consecutive PSR sessions (no EtOH or water available). The second experiment determined whether conditioned cues presented in a neutral (non-drug associated) environment could alter EtOH-seeking. Briefly, the same experimental protocol was used as in the previous experiment, except that exposure to odor cues (no odor, CS+, CS-, or CS0) occurred 30 min prior to PSR testing in a neutral environment. The third experiment examined the alterations in neuronal activity within the extended amygdala produced by exposure to CS+, CS-, or CS0. Succinctly, the experiment was conducted identically to that of the previous experiment except that brains were extracted 3 hours following the 30 min period of cue exposure (CS+, CS-, or CS0). Conditioned cues were presented in the neutral environment to eliminate the effects of goal-directed activities (lever pressing) that would occur if tested in the drug environment. Brains were analyzed using standard c-fos procedures. Areas examined were the accumbens shell (AcbSh) and core (AcbC) and the central amygdala (CeA).

Results: The data from the first experiment indicated that presentation of the CS+ increased EtOH-seeking (80% increase compared to no odor group), while the CS- prevented the expression of EtOH-seeking (65% reduction compared to no odor group). Responding in the no odor and CS0 groups were comparable. The results from the second experiment indicated that presentation of the drug cues in a neutral environment could enhance (CS+) or prevent (CS-) EtOH-seeking. In addition, the data indicated that the ability of conditioned cues to alter EtOH-seeking was greater if the cue was administered in the neutral environment than in the drug environment. The data from the third experiment indicated that the presentation of the CS+ and CS- altered the neuronal activity of the AcbSh and AcbC, but not the CeA. In the AcbSh, 30 min exposure to the CS- resulted in an increase in c-fos reactive neurons (34%), while presentation of the CS+ in the neutral environment resulted in significantly more c-fos positive neurons (78%, both compared to the CS0 group). A similar pattern of neuronal activity was observed for the AcbC (CS- 45% increase; CS+ 100% increase). Therefore, the data indicated that presentation of a

CS- and a CS+ can alter neuronal activity in both subregions of the Acb, but neuronal activity is significantly more increased following presentation of the CS+. The c-fos methodology does not allow for assessment of characteristics of c-fos positive neurons, therefore the possibility that the CS+ and CS- conditioned cues are activating distinct neurons in the AcbSh and AcbC exists.

Discussion: Overall, the data indicate that conditioned cues can enhance or prevent the expression of EtOH-seeking, and that neuronal activity in the Acb is differentially altered by presentation of these cues. Development of pharmacotherapeutics for the treatment of alcoholism could be furthered with successful treatments that reduce neuronal activity in areas associated with excitatory stimuli and enhance neuronal activity in areas associated with inhibitory stimuli. **Disclosure:** Z. Ding: None. S. Hauser: None. G. Deehan: None. W. Truitt: None. W. McBride: None. Z. Rodd: None.

17. Integrated Clinical and Translational Studies on Inflammatory Mechanisms in Late-Life Depression

Laura Dugan*, Edny Inui, Jennifer Hranilovich, Carrie Morris, Jeremy Walston, Ningying Wu, Gina D'Angelo, Tony Durbin, Yvette Sheline

University of California, San Diego, La Jolla, CA

Background: Late life depression (LLD), an important cause of worldwide disability, has been characterized in studies of phenomenology, treatment response and associated brain structural, functional and neuropsychological characteristics. There are few models that yield testable etiological hypotheses, however. An emerging concept is that inflammation is a contributing disease mechanism for many neuropsychiatric diseases, including depression. A rich literature in animal models demonstrates that activation of peripheral innate immune cytokine pathways leads to increased central proinflammatory cytokine production, and decreased neurotrophic support and neurogenesis in brain areas important to behavior and cognition. We have recently shown that the pro-inflammatory cytokine, interleukin-6 (IL-6), mediates degeneration and loss-of-phenotype of parvalbumin (PV)-positive GABAergic interneurons in hippocampus and prefrontal cortex in mouse models of aging (Dugan 2009), and schizophrenia (Behrens 2007, 2008). IL-6-deficient ($IL-6^{-/-}$) mice failed to show deficits in PV-interneurons. IL-6 effects on interneurons were mediated through activation of the superoxide-producing innate immune enzyme, NADPH oxidase (Nox2). PV-expressing interneurons are critical for normal information encoding and attention, and dysfunction of these inhibitory neurons results in cognitive deficits across domains. This led to the idea that LLD might be mediated by a similar cascade of inflammatory pathway activation and effects on inhibitory systems.

Methods: Mouse Studies: $IL-10^{-/-}$ mice, which spontaneously increases IL-6 levels starting in mid-life were used as a model of endogenous elevated IL-6. Male wild-type and $IL-10^{-/-}$ mice (12 month old, 15 each) underwent behavioral testing, including fear conditioning, and were then analyzed for PV-interneuron integrity by immunostaining, and for Nox2 activity by a number of techniques, including confocal imaging. Human Studies: Community dwelling depressed participants n=168 were matched with demographically similar comparison subjects n=50. All were assessed for depression using the Montgomery-Asberg scale (MADRS) and with the Framingham Vascular Risk Factor (VRF) scale. All subjects also received an MRI scan (Siemens 1.5 T or GE 1.5 T) and resulting MRI data were segmented using Freesurfer software. The brain volume group differences were tested after controlling for age, education, ICV, and VRF.

Results: Mouse Studies: Preliminary data demonstrate that $IL-10^{-/-}$ mice exhibit increased Nox2 activity, increased oxidative stress and loss of inhibitory interneurons in hippocampus and amygdala. This was associated with significant deficits in emotional learning, which has been attributed to both amygdala and hippocampus. Human Studies: Mean baseline MADRS scores of depressed and control cases were 26.15 (SD = 4.43) and 1.80 (SD = 1.90), respectively. Depressed

and control subjects differed significantly in the following brain volumes (mean, \pm SD), respectively): hippocampus (8072.89, \pm 1058.98) vs (8222.2, \pm 1062.78), $p = 0.001$; Amygdala (3252.36, \pm 491.92) vs (3379.34, \pm 532.5), $p = 0.001$; and frontal pole (1735.03, \pm 1393.77) vs (1882.84, \pm 541.59), $p = 0.022$.

Discussion: These results suggest that two regions, hippocampus and amygdala, which show volume loss in LLD patients, are also those selectively targeted in *IL-10*-/-mice, a model of spontaneous inflammatory pathway activation. Furthermore, *IL-10*-/-mice show deficits in emotional learning, even in the absence of stressors, and this appears in mid-life. In light of recent studies which demonstrate that psychological stress can induce hypothalamic production of IL-6, we plan to use *IL-10*-/-, wild-type, and *IL-6*-/- mice to determine whether inappropriate *IL-10*/IL-6 may enhance vulnerability to stress, and to test the role of inflammatory processes in inhibitory neuron function and integrity.

Disclosure: L. Dugan: None. E. Inui: None. J. Hranilovich: None. C. Morris: None. J. Walston: None. N. Wu: None. G. D'Angelo: None. T. Durbin: None. Y. Sheline: Part 1; Eli Lilly Speaker's Bureau.

18. Synergistic Interactions Between Prenatal Immune Challenge and Peri-Pubertal Stress in the Disruption of Adult Behavioral Functions Relevant to Schizophrenia

Joram Feldon*, Sandra Giovanoli, Urs Meyer

Swiss Federal Institute of Technology (ETH) Zurich, Scherzenbach, Switzerland

Background: Converging evidence from human epidemiological studies and parallel experimental investigations in animals indicates that prenatal exposure to infection may be a relevant environmental risk factor for schizophrenia and related disorders. However, if prenatal infection does indeed play a significant role in the etiology of schizophrenia, then it likely does so by interacting with other genetic and/or environmental susceptibility factors. Besides prenatal infection, exposure to stressful situations in per-pubertal stages of life has been repeatedly suggested to represent a significant postnatal environmental factor in the development of psychotic disorders. Against this background, the present study was designed to test the hypothesis whether prenatal viral-like immune challenge may synergistically interact with peri-pubertal stress to facilitate the emergence of schizophrenia-like behavioral abnormalities in adulthood. For these purposes, we combined a well established mouse model of prenatal (gestation day 9) immune challenge by the viral mimic Poly[I:C] (= polyriboinosinic-polyribocytidilic acid, a synthetic analogue of virus-specific double-stranded RNA) with a model of exposure to peri-pubertal stress induced by a sub-chronic variable stress protocol applied in peri-puberty (postnatal days 30 to 40). We found that peri-pubertal stress led to significant behavioral and pharmacological abnormalities specifically in animals which had been subjected to prenatal Poly[I:C]-induced immune challenge at low intensity (1 mg/kg, i.v.). These alterations included impairments in sensorimotor gating in the form of prepulse inhibition (PPI) disruption, cognitive deficits in the form of reversal learning impairment, and potentiated sensitivity to the psychotomimetic drugs amphetamine and dizocilpine (MK-801). Importantly, neither prenatal Poly[I:C] treatment at the chosen dose alone nor peri-pubertal stress alone induced such behavioral abnormalities. Hence, our initial experimental research supports the biological plausibility for synergistic interactions between prenatal immune challenge and postnatal stress in the precipitation of brain dysfunctions relevant to schizophrenia. In accordance with an environmental two-hit model of schizophrenia etiology, prenatal immune challenge may render the brain more vulnerable to postnatal stress, thereby facilitating the development of full-blown psychotic disturbances associated with schizophrenia.

Disclosure: J. Feldon: None. S. Giovanoli: None. U. Meyer: None.

19. Examination of Addictive Behavior in Rats Selectively Bred for Locomotor Response to Novelty

Shelly Flagel*, Patricia Lee, Leah Mayo, Kate Mills, Julia M. Garcia-Fuster, Peter Blandino, Sarah Clinton, Stanley J. Watson, Terry Robinson, Huda Akil

University of Michigan, Ann Arbor, MI

Background: Many people are exposed to addictive drugs throughout the course of their lives, but few become addicts. What renders some individuals more susceptible to addiction remains to be determined, but most would agree that there is no single trait underlying the disorder. We have spent the last several years studying rats that are selectively bred for differences in locomotor response to novelty and have 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 risk-taking behavior, higher susceptibility to control by reward-related cues, increased impulsivity, increased aggression and hypersensitivity of their dopamine system. Here we utilized this unique genetic animal model to determine whether bHR rats are more susceptible to addiction and to examine the relationship between some of the traits mentioned above and addiction liability.

Methods: bHR and bLR rats from generations 24, 26 and 28 were used for the current set of studies. All rats were initially screened for locomotor response to novelty and then for Pavlovian conditioned approach behavior using an autoshaping paradigm as previously described (Flagel et al, 2010). Rats then underwent jugular catheterization surgery and were trained to self-administer cocaine. During training, infusion criteria were set such that all rats received the same number of infusions and drug-cue pairings. Following the initial training phase of 2-3 weeks, rats were exposed to self-administration (SA) sessions that consisted of "drug available" periods signaled by illumination of the houselight and "no drug available" periods in which the houselight was turned off. These sessions continued for 40-60 days. Tests were conducted throughout the study to examine motivation for the drug (i.e. progressive ratio responding) and the effects of cue-removal on drug-taking behavior. Drug-induced reinstatement was examined following 1 week of abstinence and cue-induced reinstatement after 1 month of abstinence.

Results: We found that bHR and bLR rats are equally capable of acquiring drug-taking behavior, yet the pattern of drug intake for bHRs during the early phases of self-administration may be more similar to that of an addict (i.e. increased rate of drug intake). Moreover, bHR rats appear to attribute greater incentive motivational value to drug-associated cues, suggested by the fact that removal of the cue attenuates the behavior of bHRs to a greater extent than bLRs. During the latter phases of self-administration, bHRs, but not bLRs, continued to seek drug when it was no longer available. bHR rats also exhibit increased drug-seeking behavior following both drug- and cue-induced reinstatement after abstinence.

Discussion: These findings suggest that bHR rats represent those individuals at one extreme of the population that may be highly susceptible to addiction, whereas bLR rats may represent individuals at the other extreme who are resilient to the disorder. These results are especially interesting given recent studies suggesting that locomotor response to novelty is not related to addiction vulnerability in outbred rats (Deroche-Gamonet et al, 2004; Belin et al, 2008; Beckmann et al, 2010). We believe that the exaggeration of the high-responder/low-responder phenotype by the selective breeding paradigm and the additional traits that have co-segregated with these lines (e.g. impulsivity) make this an especially unique and valuable animal model to study addiction. Ongoing studies are utilizing these rat lines to examine the contribution of individual traits on addictive behavior and to uncover neurobiological correlates of these behaviors. References: Beckmann JS, Marusich JA, Gipson CD, Bardo MT. Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. *Behav Brain Res.* 2010 Jul 23. [Epub ahead of print]. Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High

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Disclosure: S. Flagel: None. P. Lee: None. L. Mayo: None. K. Mills: None. J. Garcia-Fuster: None. P. Blandino: None. S. Clinton: None. S. Watson: None. T. Robinson: None. H. Akil: None.

20. Prefrontal GABA Antagonism Models Cognitive, Behavioral and Dopaminergic Abnormalities Associated with Schizophrenia

Stan Floresco*, Maric Tse, Takeshi Enomoto

University of British Columbia, Vancouver, BC, Canada

Background: Postmortem studies of schizophrenic patients have revealed alterations in GABA-related markers in the prefrontal cortex (PFC), which is arguably one of the most reliable forms of neuropathology associated with the disorder. Despite these findings, there has been a surprising lack of preclinical research assessing how local reduction of PFC GABA activity may reflect the cognitive, behavioral and neurochemical abnormalities observed in schizophrenia.

Methods: To address this issue, we assessed the effects of pharmacological blockade of PFC GABA_A receptors in rats on executive functions and other behaviors related to schizophrenia, as well as neural activity of midbrain dopamine neurons.

Results: Blockade of PFC GABA_A receptors with bicuculline (12.5-50 ng) did not affect working memory accuracy assessed with a delayed spatial win-shift task, but did increase response latencies, resembling speed of processing deficits observed in schizophrenia. These treatments also induced perseverative and non-perseverative impairments in set-shifting, as has been observed in schizophrenic patients performing the Wisconsin Card Sorting task. Specifically, reducing PFC GABA_A transmission either prior to initial learning of a visual cue discrimination or prior to a shift to a response rule impaired set-shifting, but in qualitatively different manners. Reducing GABA activity prior to the set-shift increased non-perseverative errors, impairing the ability to acquire a novel strategy, whereas infusions prior to the initial discrimination increased perseveration during the shift. GABA_A blockade did not affect acquisition of either visual-cue or response discrimination, retrieval of visual-cue discrimination, or reversal learning, suggesting that deficits in set-shifting were not due to non-specific impairments in learning or flexibility. Latent inhibition of aversive conditioning was unaffected by bicuculline infusions prior to the pre-exposure/conditioning phases, suggesting that impairments in "learned irrelevance" observed in schizophrenia may not be related to reduced PFC GABA activity. On the other hand, GABA_A blockade increased locomotor activity, and showed synergistic effects with a subthreshold dose (0.25 mg/kg) of amphetamine. Furthermore, neurophysiological recordings from midbrain dopamine neurons revealed that reducing medial PFC GABA activity selectively increased phasic burst firing of ventral tegmental area dopamine neurons, without altering their overall population activity.

Discussion: These results suggest that PFC GABA hypofunction may be a key contributing factor to deficits in speed of processing, cognitive flexibility and enhanced phasic dopamine activity observed in schizophrenia. As such, this approach may represent a relatively straightforward animal model for the cognitive deficits associated with schizophrenia, that in turn may be useful for preclinical assessments of novel pharmacotherapeutic treatments for these symptoms.

Disclosure: S. Floresco: Part 1; Dainippon Sumitomo Pharma Co. M. Tse: None. T. Enomoto: Dainippon Sumitomo Pharma Co.

21. Loss of mPFC NMDAR Function Impairs Attention in Association with Loss of Interneuron Parvalbumin and GAD67 Expression: Are the Young Most Vulnerable?

Margarita Behrens, Janet Finlay, Gabe Puttrese, Nanda Regmi, Robert Greene*

Western Washington Univ, Bellingham, WA, UT Southwestern, Dallas, TX

Background: A generalized dysfunction of glutamate N-methyl-D-aspartate receptors (NMDARs) has been shown to decrease the number of parvalbumin and GAD67 expressing neurons in the medial prefrontal cortex (mPFC) of mice, a pathological finding associated with schizophrenia that may contribute to cognitive deficits of schizophrenia. In mice and culture preparations, the loss of expression (evoked by ketamine treatment) is due to a neuroinflammatory-like response mediated by an increase in IL6, increasing NADPH oxidase activity, and causing oxidative damage. We have reported that a localized deletion of a functionally requisite exon for the NR1 subunit of the glutamate NMDA receptor in the PFC of adult (60 day old) mice disrupts sustained attention as assessed with 5-choice serial reaction time test. Since the onset of schizophrenia-associated morbidity is usually around the time of late adolescence, the possibility of developmental sensitivity to this loss of NMDAR function exists. More specifically, the effect of loss of NMDAR activity in the mPFC of mice aged 60 days compared to 30 days on mPFC morphology needs characterization, ultimately, in association with mPFC-dependent cognition. Because dopamine terminals may be especially sensitive to oxidative insult, tyrosine hydroxylase (TH) terminals in mPFC may be examined for evidence of developmentally sensitive damage.

Methods: Localized gene deletions were induced using an AAV-Cre vector together with floxed NR1 transgenic mice (a gift from Tonegawa lab). The deletions were induced in mPFC of 60 day old mice (6od; n = 3) or 30 day old mice (3od; n = 5) for comparison with control, AAV-mediated, expression of GFP in mPFC. All animals were sacrificed at age day 80 for immunohistochemical characterization.

Results: In both groups, there were fewer Prv labeled neurons compared to controls and the difference was significantly larger in the 3od compared to the 6od ($p < 0.05$). The 6od had fewer Prv+ neurons than controls but this was only a trend. GAD67 was also reduced in the remaining Prv+ neurons of the AAV-injected animals. Alternate sections were stained for TH in these animals. In the 3od mPFC, TH positive terminals appeared to be fewer in number and were more globular-like in appearance compared to 6od or control. The 3od TH was also notable for a lack of fine threadlike staining that was prominent in the 6od or control sections. Quantification of the TH immunohistochemical findings will be discussed.

Discussions: These findings suggest that NMDA receptor dysfunction in the mPFC induces a developmentally sensitive disruption of interneuron phenotype and an abnormal morphology of TH+ terminals in the mPFC. Our earlier findings of attentional deficits in mice with the same loss of NMDAR function in mPFC suggest that the morphological deficits reflect a functional association of the morphological and behavioral findings. Further, the morphological changes show an adolescent vulnerability to loss of NMDAR function in mPFC. We are now examining the developmental sensitivity of the sustained attention impairment. Supported by 5P50 MH060450-08 and RO1 MH080297-04 to RWG and WWU Research and Sponsored Projects to JMF.

Disclosure: M. Behrens: None. J. Finlay: None. G. Puttrese: None. N. Regmi: None. R. Greene: None.

22. Smaller Dentate Gyrus Volume and Increased Neural Progenitor Cell Proliferation in Alcohol Dependent Subjects

Maura Boldrini, Kelly Burke, Sharlene Leong, J. John Mann, Victoria Arango*, Mark Underwood

Columbia University, New York, NY

Background: The effects of alcohol on hippocampal neurogenesis are complex, and can be different depending on species, dose, pattern of

intake and time following brain alcohol exposure. Alcoholics exhibit neurological symptoms considered sequelae of hippocampal pathology. Chronic alcohol self-administration reduces hippocampal neurogenesis in mice and is reversed by fluoxetine. Abstinence also leads to less neurogenesis. Chronic treatment with desipramine during abstinence prevents this reduction. In the rat, the number of mitotic cells and immature neurons in the dentate gyrus (DG) of the hippocampus was decreased immediately after alcohol exposure (day 0), followed by a rebound on day 3, returning to normal levels on days 7 and 21. We sought to determine whether neurogenesis, as measured by the number of neural progenitor cells (NPCs) and mitotic cells in DG is altered in subjects with alcohol dependence (ALC) compared to non-psychiatric controls (NC).

Methods: We studied matched NC ($n=9$, 19-72y of age, 6 males, 3 females, with 9.5-19 h postmortem interval [PMI], pH 5.84-6.87) and ALC ($n=9$, 24-67y old, 6 males, 3 females, PMI 9-24 h, pH 6.15-6.90, blood alcohol levels from 0.01-0.34%). All subjects underwent toxicological and neuropathological screens and a psychological autopsy generating DSM Axis I and II diagnoses and medical illness, treatment, and family history. All cases died suddenly and had no neurological disease. Immunocytochemistry was performed to identify NPCs (nestin-IR) and mitotic cells (Ki67-IR). Cell number and DG volume were quantified by stereology. Cell number and DG volume were assessed in alcoholics in a region-specific manner, analyzing the rostral, middle and caudal DG separately.

Results: In NC there were 565 ± 483 NPCs and $2,438 \pm 1,548$ mitotic cells in the DG, which did not differ statistically from ALC who had $2,857 \pm 5,679$ NPCs ($t = -1.211$, $df = 15$, $p = 0.245$) and $1,971 \pm 1,418$ mitotic cells ($t = 0.943$, $df = 16$, $p = 0.515$). However, subjects with alcohol dependence and high (0.2% and above) blood alcohol level (H-ALC) compared to ALC with low alcohol level (L-ALC) and NC had: (1) more NPCs in the entire DG (NC: 565 ± 161 , L-ALC: 343 ± 132 ; H-ALC: $7,049 \pm 4850$, $F = 5.205$, $df = 2,16$, $p = 0.020$; H-ALC vs L-ALC: $p = 0.031$; H-ALC vs NC $p = 0.023$); (2) higher NPC density (cells per mm³) in the entire DG (NC: 7 ± 2 , L-ALC: 6 ± 3 ; H-ALC: 114 ± 57 , $F = 10.044$, $df = 2,16$, $p = 0.002$; H-ALC vs L-ALC: $p = 0.004$; H-ALC vs NC $p = 0.002$); and (3) higher NPC density (cells per mm³) in the rostral DG (H-ALC: 127 ± 65 ; L-ALC: 0 ± 0 ; NC: 10 ± 6 , $F = 4.807$, $p = 0.043$; H-ALC vs L-ALC: $p = 0.06$; H-ALC vs NC $p = 0.05$). Blood alcohol level was positively correlated with the total number (Pearson Correlation [PC] = 0.578, $p = 0.019$) and density (PC = 0.627, $p = 0.009$) of nestin-IR NPCs in the DG. There was also a trend for a correlation of alcohol blood level with NPC density (PC = 0.623, $p = 0.054$) and number (PC = 0.595, $p = 0.053$) in the rostral DG. ALC had a smaller DG volume (mm³) than NC (NC: 110.41 ± 15.85 ; ALC: 64.15 ± 10.21 ; $t = 2.383$; $df = 15$; $p = 0.031$), with no difference between H-ALC (61.43 ± 13.15) vs L-ALC (68.66 ± 19.51), suggesting that the greater number of NPC and mitotic cells in ALC than NC was not due to a larger DG volume. Increasing age was correlated with fewer Ki67-IR cells in the head of the DG (-0.709, $p = 0.010$). In ALC, the correlation showed a trend to significance ($p = 0.069$) and there was no correlation in the NC group ($p = 0.113$). There was no relationship between DG volume, cell number or density and alcoholism severity (SCID-alc score) or duration, PMI or tissue pH.

Discussion: NPC number is higher in ALC subjects with high blood alcohol level compared to ALC with low blood alcohol levels or NC, suggesting that alcohol can have an acute effect of increasing NPC proliferation. However, since the DG volume is smaller in ALC, the acute alcohol effects do not appear to lead to cell maturation and survival. In the rat, neurogenesis decreases immediately after alcohol exposure and it shows a burst after two days, returning to normal levels after one week. Studies in mice show decreased neurogenesis with chronic alcohol exposure. Differences between animals and humans and the effect(s) of alcohol could be the result of differences in the cognitive effects of alcohol, the effects on neurotransmitters or the impact of stress or environment. Rodents and humans might also differ in alcohol metabolism and the timing of its effects on cell replication. Additional studies are needed to determine the possible

mechanisms mediating the effects of alcohol on cell proliferation and possibly neurogenesis in human.

Supported by MH40210, MH62185, MH64168, MH083862, AA09004, AA11293

Disclosure: M. Boldrini: None. K. Burke: None. S. Leong: None. J. Mann: Part 4; GlaxoSmithKline, Novartis. V. Arango: None. M. Underwood: None.

23. Abnormal Cystine-Glutamate Exchange by System xc- in Neurodevelopmental Models of Schizophrenia

Victoria Lutgen, Aric Madayag, Peter Dietrich, Krista Qualmann, David Baker*

Marquette University, Milwaukee, WI

Background: Reduced cystine-glutamate exchange by system x_c^- has been implicated in schizophrenia. First, increased cystine-glutamate exchange following administration of the cysteine prodrug N-acetylcysteine has been shown to reverse abnormal rodent behaviors produced by acute phencyclidine treatment in preclinical studies and negative symptoms of schizophrenia in a phase II clinical trial. Second, protein levels measured in post mortem tissue revealed significant changes in the level of xCT, the active subunit for system x_c^- , in dorsolateral prefrontal cortex obtained from schizophrenic patients. As a result, the current study sought to determine whether altered system x_c^- activity may be linked to the pathology of schizophrenia by assessing the status of cystine-glutamate exchange and the impact of increased system x_c^- on abnormal behaviors in neurodevelopmental models of schizophrenia.

Methods: Timed pregnant Sprague Dawley rats were given an acute injection of vehicle or methylazoxymethanol (MAM; 22 mg/kg, IP; gestational day 17) or underwent a variable stress procedure (gestational days 14-22). Following vaginal birth all mothers and offspring were left undisturbed until weaning on postnatal day 22. Male offspring were single caged for testing as adults (PND 60+). System x_c^- -activity was assessed by measuring tissue levels of glutathione (assessed using HPLC with EC detection - Decade II, reactor cell, -1.4V; Flex Cell + 0.65V) or by measuring cystine uptake (^{14}C -cystine applied to tissue punches at 0.3 microM for 0-20 min) in prefrontal cortical tissue punches obtained from adult offspring. Behavioral assays included set shifting and novel object recognition. For set shifting, rats were trained and tested on a four-arm cross maze (60 X 20 X 12 inches). Following training to habituate the rats to receiving and consuming food in the maze, rats underwent three days of testing. On day 1, rats were trained to enter the arm containing a visual cue to obtain a food reinforcer. On day 2, rats were reinforced upon entering the arm of the maze opposite to their turn bias established during training regardless of the placement of the visual cue. The visual cue was placed with equal frequency randomly in each arm. On the third day, rats were reinforced when turning into the arm that represented their turning bias. For object recognition, rats were placed in a black open field box (16" X 19.5" X 21"). The objects to be discriminated were a white plastic cylinder (2.25" x 1.875"), and a blue and green Lego cube (2.5" x 1.5" x 1.25"). During the experimental session, two trials were run on each rat. In the preliminary trial the rats were placed in the box and given 3 minutes to explore two identical objects. Sixty minutes later, rats were permitted to explore a "familiar" or the "novel" object for three minutes. During the test days for both novel object recognition and set shifting, rats received injections of vehicle or N-acetylcysteine (60 mg/kg, IP) sixty min prior to testing.

Results: Both MAM administration (gestational day 17) or prenatal stress (gestational days 14-22) resulted in abnormal activity of system x_c^- when assessed in tissue punches obtained from adult offspring. Specifically, reduced ^{14}C -cystine uptake was evident in prefrontal cortical tissue punches obtained from rats that had received either MAM administration ($t_4 = 4.473$, $p < .05$) or prenatal stress ($t_{13} = 2.384$, $p < .05$). Further, reduced levels of glutathione was obtained in tissue punches obtained from rats that underwent prenatal stress ($t_{23} = 2.4$, $p < .05$); a trend toward reduced glutathione was evident in rats that had

received MAM treatment (t -test, $p > .05$). MAM-treated adult offspring exhibited deficits in set shifting (day 3; reversal learning; Dunnett's T , $p < .05$ between veh/veh and MAM/veh subjects), and object recognition (Dunnett's T , $p < .05$ between veh/veh and MAM/veh subjects) that was not evident when subjects received acute N-acetylcysteine treatment (Dunnett's T , $p > .05$ between vehicle/NAC and MAM/NAC groups).

Discussion: Our data show that reduced system xc- activity is evident in prefrontal cortical tissue slices obtained from adult offspring that had received MAM treatment on gestational day 17 or prenatal stress on gestational days 14–22, two neurodevelopmental models of schizophrenia. Moreover, behavioral deficits observed in adult offspring that had received MAM treatment in utero were reversed by acute N-acetylcysteine when administered prior to behavioral testing. Collectively these data implicate diminished system xc- activity in abnormal behaviors associated with neurodevelopmental models of schizophrenia.

Disclosure: V. Lutgen: None. A. Madayag: None. P. Dietrich: None. K. Qualmann: None. D. Baker: Part 1; Promentis Pharmaceuticals. Part 2; Promentis Pharmaceuticals. Part 3; Promentis Pharmaceuticals. Part 4; Promentis Pharmaceuticals.

24. Ovarian Steroids Decrease CRF-R1 Expression on Serotonin Neurons in the Dorsal Raphe of Macaques

Rachel L. Sanchez, Cynthia L. Bethea*

Oregon National Primate Research Ctr, Beaverton, OR

Background: A significant number of postmenopausal women report increased anxiety and vulnerability to stress, which has been linked to decreased secretion of ovarian steroids. Communication between the corticotropin releasing factor (CRF) system and the serotonin system is well established and may play an important role in stress sensitivity or resilience. Previous studies have shown that CRF acting at CRF type 1 receptors (CRF-R1) decreases serotonin neurotransmission. We questioned whether ovarian steroids regulate the expression of CRF-R1 on serotonin neurons in the dorsal raphe of nonhuman primates.

Methods: Ovariectomized rhesus macaques were treated with placebo, E alone for one month, or E for one month supplemented with P for the last 2 weeks. Using Taqman microfluidic custom expression cards (ABI), quantitative (q)RT-PCR for CRF-R1 expression was performed on hemi-midbrain blocks ($n = 4$ /group) and pools of laser captured serotonin neurons ($n = 3$ /group). Immunocytochemistry for CRF-R1 and stereological analysis were also performed at 5 levels of the dorsal raphe ($n = 3$ animals/group). ANOVA followed by Student-Newman Keuls posthoc test were used for statistical comparison.

Results: E ± P treatment significantly decreased CRF-R1 mRNA in hemi-midbrain blocks and in laser captured serotonin neurons (ANOVA $p < 0.0014$ and 0.0124 , respectively). E ± P treatment also significantly decreased CRF-R1 immunostaining (pixel area) in the dorsal raphe nucleus (ANOVA $p < 0.03$).

Discussion: These data add to the evidence that the CRF and serotonin systems are inextricably linked, and show that ovarian steroids reduce the expression of the angiogenic CRF-R1 receptor on serotonin neurons in nonhuman primates. The reduction in CRF-R1 would reduce the inhibitory action of CRF on serotonin neurons, thereby increasing serotonin neurotransmission. The increase in serotonin could relieve anxiety and increase stress resilience. Supported by MH62677 to CLB and RR00163 for support of ONPRC.

Disclosure: R. Sanchez: None. C. Bethea: None.

25. Integrin Modulates Responsiveness to Selective Serotonin Reuptake Inhibitors *In Vivo*

Michelle Carter, Jeremy Veenstra-VanderWeele,
Ana Carneiro*

Vanderbilt University School of Medicine, Nashville, TN

Background: The structural and functional alterations in synaptic connections leading to impaired neuronal communication are thought

to be involved in the manifestation of neuropsychiatric disorders. Integrins are differentially expressed in the brain, where they modulate the assembly of synapses through the recruitment of scaffolding proteins that modulate receptor localization and synaptic vesicle function. The integrin beta 3 gene (ITGB3) has been associated with autism, with a strong synergistic association with the serotonin transporter gene (SLC6A4). Although common, the molecular consequence of most ITGB3 polymorphisms remains unknown. Here we utilize mouse models to determine the role of integrin beta3 on the modulation of the serotonergic system, specifically by influencing the function of presynaptic serotonin transporters (SERT). SERTs are responsible for rapid 5-HT inactivation at synapses and represent a major mechanism for regulating extracellular levels of 5-HT.

Methods: To model human polymorphisms leading to loss-of-function of integrin beta3 we utilized heterozygous Itgb3 mice. Neurochemical characterization consisted on monoamine reuptake in synaptosomal preparations and radiolabeled ligand binding for quantification of 5-HT receptors and transporters. Behavioral characterization included, but was not restricted to, behaviors influenced by selective 5-HT reuptake inhibitors (SSRIs): overall locomotor activity, thigmotaxis, forced swim test (FST) and tail suspension tests (TST). To measure SSRI responsivity, racemic citalopram hydrobromide was administered by intraperitoneal injection in a volume of 0.01 mL/g body weight and the dose was 0 , 2 , 5 , or 10 mg/kg calculated as the weight of the base. Each mouse was tested four times, with 1 week between testing. A counterbalanced design was used, where half of the animals of each genotype received 10 one order (0 , 10 , 2 , 5 mg/kg) and the other half received the other order (10 , 0 , 5 , 2 mg/kg).

Results: In the brain, integrin beta3 localizes to growth cones of 5-HT neurons and interacts with SERT in mouse midbrain presynaptic membranes. 5-HT saturation kinetics revealed significant reductions in SERT catalytic properties in Itgb3 $+/-$ mice, when compared to control littermates ($K_m: \text{wt} = 198$. Itgb3 $+/- = 28.80$; $V_{max}: \text{wt} = 696.70$, Itgb3 $+/- = 343.30$). We found no significant genotype effects in the open field, thigmotaxis or basal immobility in the TST. In minutes 3–6 of the FST, we observed significant reductions in basal immobility time of Itgb3 $+/-$ mice ($\text{wt} = 93.84$; Itgb3 $+/- = 39.57$). Itgb3 $+/-$ also exhibited a significant decrease in fecal boli, a sign of diminished anxiety ($\text{wt} = 6.5$; Itgb3 $+/- = 4.7$). Itgb3 $+/-$ mice consistently exhibited increased sensitivity to citalopram in both TST and FST. After administration of 10 mg/kg citalopram, both genotypes displayed decreased immobility as expected. However, a lower dose of citalopram (5 mg/kg) produced a significant reduction in immobility in Itgb3 $+/-$ mice, but not in wild type littermates in both FST and TST. We also observed significant increases in fecal boli in Itgb3 $+/-$ mice after citalopram administration. These changes do not appear to arise from perturbed sensitivity of SERT to citalopram as the SSRI displayed equivalent potency for inhibition of 5-HT transport in synaptosomes from Itgb3 $+/-$ and wild type littermates.

Discussion: While the complete blockade of SERT by SSRIs represent a widely utilized treatment for several neuropsychiatric disorders, the modulation of specific SERT populations (e.g. high-capacity transporters) may improve treatment efficacy and outcome. The development of novel therapies for mood disorders will likely advance as the understanding of gene and protein networks involved in 5-HT dysfunction progresses. The studies presented here reveal an unexpected role of integrins in serotonergic homeostasis, more specifically, in the regulation of presynaptic SERTs. In mouse models, integrin beta3 haploinsufficiency influences SSRI responsivity and may influence antidepressant therapy in human populations. Some promoter polymorphisms in ITGB3 (rs62074393 and rs7208055) have allele frequencies of 50% and show reductions in beta3 expression *in vitro*. Our Itgb3 $+/-$ mice recapitulate these polymorphisms may represent important markers for the development of personalized medicine. Finally, the direct, physical interaction between integrin beta3 and SERT reveals a novel target for the development of pharmacotherapies targeting the 5-HT system. Support: NIH MH081066 (JV), NARSAD (AMDC).

Disclosure: M. Carter: None. J. Veenstra-VanderWeele: None. A. Carneiro: None.

26. The Mood Stabilizers Valproic Acid and Lithium Enhance Mesenchymal Stem Cell Migration: Implications for Cell Replacement Therapies in Brain Disorders

Li-Kai Tsai, Yan Leng, Zhifei Wang, Peter Leeds, De-Maw Chuang*

NIMH, NIH, Bethesda, MD

Background: Mesenchymal stem cells (MSCs) exhibit high potential for the therapy of several human diseases. In addition to mechanisms for cell replacement, MSCs possess therapeutic effects for immunomodulation, trophic action, neuroprotection, and stimulation of angiogenesis. However, the effectiveness of MSC transplantation has been hampered by the relatively poor migratory capacity of these cells toward disease target sites. Valproic acid (VPA), a mood stabilizer and anti-seizure agent which can enhance GABA transmission, reduce glutamate and dopamine transmission, as well as reduce infarct volume, suppress neuroinflammation, induce neurogenesis, and improve behavioral performance in a stroke model through histone deacetylase (HDAC) and glycogen synthase kinase-3 (GSK-3) inhibition, enhances the expression of CXC chemokine receptor 4 (CXCR4) in hematopoietic stem cells. CXCR4 is an α -chemokine receptor specific for stromal cell-derived factor 1 α (SDF-1 α), a molecule endowed with potent chemotactic activity first noted in lymphocytes. Because MSC migration is also mediated by SDF-1 α /CXCR4 interaction, it is possible that inducing CXCR4 expression may promote the migratory potential of MSCs. However, whether VPA up-regulates CXCR4 in MSCs and enhances MSC migratory capacity remains unclear. Lithium, a major mood stabilizer, through inhibition of GSK-3 β activates the Wnt downstream signaling pathway which is then involved in the development, adult tissue homeostasis, regulation of MSC migration, and inhibition of GSK-3 β that leads to up-regulation of target genes. Lithium also enhances levels of GABA, acetylcholine, and 5-hydroxytryptamine in the brain, inhibits dopamine-mediated neurophysiological functions, and like VPA, displays neuroprotective effects in multiple *in vivo* and *in vitro* experimental settings. Lithium treatment has been shown to enhance the migratory capacity of MSCs, but the underlying mechanisms remain elusive. This study investigated whether VPA and lithium treatment could enhance MSC migration and, if so, the underlying mechanisms involved.

Methods: Cryopreserved rat MSCs were purchased from Cell Applications (San Diego, CA) and used in the fifth passage of cultivation. Cells were harvested after 4 days of treatment for subsequent experiments except immunocytochemistry, which was performed after 2-3 days. Data were obtained by immunocytochemistry, real-time PCR, western blot and chromatin immunoprecipitation. Cell migratory ability and chemotaxis were measured by a modified Boyden chamber with polycarbonate membrane filters. MMP enzymatic activity was measured by zymography. GSK-3 β Mission® small hairpin RNA (shRNA) Plasmids and non-targeting shRNA control vector (Sigma-Aldrich) were used for GSK-3 β knockdown studies.

Results: Our results showed that acute (three-hour) treatment of MSCs with a relatively high concentration (2.5 mM) of VPA markedly increased transcript and protein levels of CXCR4. This VPA-induced CXCR4 expression required HDAC inhibition, including the HDAC1 isoform, and involved histone hyperacetylation at the CXCR4 gene promoter region. Of interest, SDF-1 α -mediated MSC migration was enhanced by VPA treatment, and this was completely blocked by AMD3100, a CXCR4 antagonist. MSC treatment for one day with lithium (2.5 mM) selectively elevated transcript and protein levels of matrix metalloproteinase-9 (MMP-9) and its enzymatic activity. These effects were mimicked by GSK-3 β inhibition or gene silencing. In a Boyden Chamber migration assay, lithium treatment also potentiated

SDF-1 α -dependent MSC migration across the extracellular matrix, and this could be suppressed by either doxycycline or GM6001, two MMP-9 inhibitors. Combined VPA and lithium treatment further increased MSC migration. Thus, our results showed that VPA and lithium could stimulate MSC migration via distinct targets and mediators: HDAC-CXCR4 and GSK-3 β -MMP-9, respectively.

Discussion: MSC-based therapy is beneficial for the treatment of several human diseases including cerebral ischemia, myocardial infarction, amyotrophic lateral sclerosis, spinal cord injury, multiple sclerosis, and musculoskeletal disorders. The results of the present study suggest that VPA, through HDAC-CXCR4, and/or lithium, through GSK-3 β -MMP-9, can be used as priming drugs to enhance the migratory capacity or homing effects of MSCs toward disease target sites after transplantation, which might expand the clinical use of these two mood stabilizers in neurodegenerative and neuropsychiatric conditions.

Disclosure: L. Tsai: None. Y. Leng: None. Z. Wang: None. P. Leeds: None. D. Chuang: None.

27. Orexin Receptor Antagonists Attenuate Alpha2-Adrenergic Receptor-Independent Effects of Yohimbine on Extinction of Cocaine Reward and Excitatory Transmission in the Bed Nucleus of the Stria Terminalis

Kelly Conrad*, Adeola Davis, Sam Saleh, Craig Lindsley, Danny Winder

Vanderbilt University Medical Center, Nashville, TN

Background: Yohimbine is a frequently used pharmacological stressor that is thought to act via its inhibition of noradrenergic autoreceptors (alpha2-adrenergic receptors, alpha2-ARs). Previous research has demonstrated that yohimbine 1) impairs extinction of cocaine conditioned place preference (cocaine CPP) in C57Bl/6J as well as alpha2a-AR knockout mice, 2) impairs extinction of cocaine self administration in rats, and 3) increases alcohol self administration. In acute brain slices containing the bed nucleus of the stria terminalis (BNST), yohimbine has been shown to depress excitatory transmission. However, these effects of yohimbine are intact in alpha2a-AR knockout mice and not fully mimicked by the more selective alpha2-AR antagonist atipamezole, suggesting that they are mediated by targets other than alpha2-ARs. Recent studies using yohimbine have proposed an interaction between the noradrenergic and orexinergic systems. Thus, the purpose of this study was to assess the potential role of orexin receptors in the actions of yohimbine on reward-related behavior as well as synaptic function in the BNST.

Methods: Wildtype and alpha2a-AR knockout mice were trained in the cocaine CPP paradigm and the effects of the orexin receptor antagonist, SB-334867, on yohimbine-impaired extinction were investigated. The effects of SB-334867 as well as a newly synthesized specific orexin receptor antagonist, 2-[4-(5-methyl-2-(2H-1,2,3-triazol-2-yl)benzoyl]-1,4-diazepan-1-yl]quinazoline (MTBDQ), on yohimbine-induced depression of excitatory transmission in the BNST were also examined.

Results: Our results confirm that yohimbine-induced impairment of extinction of cocaine CPP was enhanced in alpha2a-AR knockout mice. Our results also indicate SB-334867 attenuated yohimbine-induced impairment of extinction of cocaine CPP in wildtype and alpha2a-AR knockout mice. In addition, both SB-334867 and MTBDQ had no effect on excitatory transmission but blocked yohimbine-induced depression in BNST field potential recordings.

Discussion: Taken together, these results confirm a previous study suggesting that Yohimbine acts in an alpha2-ARs-independent manner in mediating stress-induced impairment of extinction of cocaine CPP and suggest that the orexin signaling system may be a direct target for the actions of yohimbine on cocaine related behaviors and excitatory transmission in the BNST.

Disclosure: K. Conrad: None. A. Davis: None. S. Saleh: None. C. Lindsley: None. D. Winder: None.

28. Histone deacetylase 5 (HDAC5) Limits Cocaine Reward Through a Novel Signaling Mechanism Involving Cyclin-Dependent Kinase 5 (Cdk5), cAMP, and Protein Phosphatase 2A (PP2A)
 Makoto Taniguchi, Maria Carreira, Benjamin Zirlin, Christopher Cowan*

UT Southwestern Medical Center, Dallas, TX

Background: Repeated exposure to cocaine induces molecular and cellular adaptations in brain reward circuit neurons that promote or antagonize behavioral responses in animal models of addiction. Recently, epigenetic mechanisms, such as gene regulation by histone deacetylases (HDACs), have emerged as key modulators of behavioral responses to repeated cocaine exposure, but whether or how HDACs are regulated by cocaine remains poorly understood.

Methods: We utilized numerous *in vivo*, *in vitro* and neuronal cell culture approaches to explore how acute and chronic cocaine regulates the function and role of HDAC5 in the adult mouse striatum. Using novel point mutants, phospho-site specific antibodies, recombinant viruses, and biochemical manipulations of striatal tissue and cultured neurons, we explored the regulation and function of HDAC5 by cocaine and cAMP signaling pathways.

Results: We found that cocaine and cAMP signaling induce the nuclear import of the class IIa HDAC, HDAC5, in the striatum, which functions to dampen the rewarding effects of cocaine. However, repeated cocaine exposure reduces this effect through upregulation of cyclin-dependent kinase 5 (Cdk5)-dependent phosphorylation of HDAC5 at a novel site (Ser279) within the nuclear localization sequence. We show that phosphorylation of HDAC5 Ser279 reduces nuclear import and accelerates nuclear export of HDAC5, which serves to block the nuclear accumulation of HDAC5 after repeated cocaine exposure and to abrogate the ability of HDAC5 to reduce cocaine reward in cocaine-experienced mice. In contrast to chronic cocaine, we find that “acute” cocaine exposure in drug-naïve mice induces rapid nuclear import of striatal HDAC5 through a mechanism involving cAMP- and protein phosphatase 2A (PP2A)-dependent dephosphorylation of HDAC5 Ser279. Importantly, we find that only the dephosphorylated form of HDAC5 Ser279 in the nucleus accumbens represses cocaine reward.

Discussion: Taken together, these findings reveal a novel regulatory mechanism by which HDAC5 limits cocaine reward and a signaling adaptation to chronic cocaine (i.e. increased P-Ser279) that antagonizes that effect. As such, dephosphorylation of HDAC5 Ser279 may represent a new therapeutic target for the treatment of drug addiction.

Disclosure: M. Taniguchi: None. M. Carreira: None. B. Zirlin: None. C. Cowan: None.

29. Loss of Association Between PGC-1Alpha and its Putative Targets in the Dorsolateral Prefrontal Cortex of Patients with Schizophrenia
 Rita Cowell*, J. Matt Rutherford, Elizabeth Lucas, Vahram Haroutunian, James H. Meador-Woodruff

University of Alabama at Birmingham, Birmingham, AL

Background: Prefrontal cortical GABAergic dysfunction is a hallmark of schizophrenia. In particular, markers of GABAergic function (e.g. parvalbumin (PV) and glutamic acid decarboxylase 67) are reduced in the cortex of patients with schizophrenia. Our lab recently determined that cortical expression of PV requires the transcriptional coactivator PGC-1alpha (peroxisome proliferator activated receptor gamma coactivator 1 alpha), a gene recently associated with schizophrenia. We hypothesize that PGC-1alpha is important for the regulation of PV and other interneuron-specific genes and that abnormalities in the PGC-1alpha pathway contribute to alterations in PV expression in schizophrenia.

Methods: To begin to investigate these hypotheses, we evaluated the relationship between PGC-1alpha and PV mRNA expression in cortical samples from patients with schizophrenia and a comparison group using real-time RT-PCR. Furthermore, we performed preliminary

luciferase reporter assays to elucidate the mechanisms by which PGC-1alpha controls PV expression.

Results: Transcriptional analyses of human dorsolateral prefrontal (DLPFC) and anterior cingulate cortex ($n=23-25/\text{group}$; Mt. Sinai Collection) revealed that PGC-1alpha and PV transcript levels were not different between control and schizophrenia subjects in this cohort. However, PGC-1alpha and PV transcript expression was strongly correlated in the comparison group (C, $r=0.54$), and this correlation was absent in patients with schizophrenia (SZ, $r=0.03$). Strong correlations were also found between PGC-1alpha and other putative targets in DLPFC of control subjects (targets recently identified in our lab by microarray, see Lucas et al.), and these correlations were lost in patients with schizophrenia. These targets included synaptotagmin II (C, $r=0.90$; SZ, $r=0.18$), complexin I (C, $r=0.90$; SZ, $r=0.36$), and neurofilament heavy chain (C, $r=0.45$; SZ, $r=-0.03$), three proteins involved in synaptic transmission and structural integrity in GABAergic neurons. Interestingly, preliminary luciferase assays revealed a 150 bp region of the PV promoter required for PGC-1alpha-mediated induction that includes a consensus binding site for a transcription factor involved in GABAergic specification.

Discussion: Collectively, these data suggest that PGC-1alpha controls a subset of genes important in interneuron function and specification and that this transcriptional program is compromised in schizophrenia. Further investigation of the mechanisms by which PGC-1alpha regulates its target genes may reveal critical targets for ameliorating interneuron dysfunction.

Disclosure: R. Cowell: None. J. Rutherford: None. E. Lucas: None. V. Haroutunian: None. J. Meador-Woodruff: Part 2; ACNP, editor-in-chief of Neuropsychopharmacology.

30. Regulation Of Hippocampal Neurogenesis Through IL-1beta And Inflammasome Proteins

Fulton Crews*

University of North Carolina, Chapel Hill, NC

Background: Adult hippocampal neurogenesis is implicated in the regulation of mood and cognition. *In vivo*, exercise and anti-depressants increase neurogenesis, whereas ethanol, other addictive drugs, inflammation and aging reduce neurogenesis. To investigate the molecular mechanisms of these changes in neurogenesis we developed a hippocampal-entorhinal cortical (HEC) slice culture to study hippocampal neurogenesis *in vitro*.

Methods: HEC slices were prepared from P7 rats and cultured for 2-4 weeks with conditions optimized for neurogenesis. Expression of doublecortin (DCX), a marker of new immature neurons, and Ki67 a marker of proliferating neuroprogenitors were used to quantify neurogenesis. RTPCR and ELISA assessed mRNA and protein respectively.

Results: Ethanol treatment of HEC slices for 4 days reduced neurogenesis by 50-70%, similar to *in vivo* findings that associate with negative affect behaviors. In addition, ethanol treatment increased mRNA for a variety of innate immune genes, TNFalpha, MCP-1, IL-1beta and IL6. IL-1beta is formed by an inflammasome protein complex and inflammasome proteins NALP1, NALP3, and caspase-1, were all increased in neurons and astrocytes by ethanol treatment. Exogenous IL-1beta, TNFalpha or MCP1 reduce neurogenesis. Neutralizing antibodies to TNFalpha and MCP1 had small effects, whereas neutralizing antibodies to IL-1beta increased neurogenesis and completely reversed ethanol inhibition of neurogenesis. IL-1 receptor antagonist, IL-1RIa, also increased neurogenesis suggesting that IL-1beta mediates ethanol inhibition of neurogenesis. A number of other drugs known to improve negative affect were found to increase neurogenesis and reverse ethanol inhibition of neurogenesis. These include the anti-depressants imipramine and fluoxetine, drugs that blunt innate immune activation, e.g. rolipram, butylated hydroxytoluene, and VEGF, as well as the histone deacetylase inhibitors, TSA and VPA, and the mGluR5 agonist DHPG.

Discussion: Inflammasome formation produces IL1beta that activates further innate immune genes and reduces neurogenesis. Agents that reduce IL1beta increase neurogenesis. Taken together these findings suggest that inflammasome- IL1beta induction inhibits neurogenesis. Innate immune gene induction and loss of neurogenesis are associated with negative affect. Agents that reverse ethanol induction of IL1beta and stimulate neurogenesis are associated with reversing depression and improving mood. These findings suggest inflammasome induction and neurogenesis contribute depression and negative affect. Stimulation of neurogenesis and reduced inflammasome-IL1beta contribute to the mechanisms of antidepressant therapy (supported by NIAAA).

Disclosure: F. Crews: None.

31. Peptide Disruption of the Interaction Between the Serotonin (5-HT) 5-HT_{2C} Receptor (5-HT_{2C}R) and Protein Phosphatase and Tensin Homologue Deleted on Chromosome 10 (PTEN) Is Functionally Important to the 5-HT_{2C}R Signalingosome

Kathryn Cunningham*, Fernanda Laezza, Andrew McGinnis, Patricia Seitz, Alexander Shavkunov, Anton Agarkov, Neli Panova-Elektronova, Noelle Anastasio, Latham Fink, Cheryl Watson, Amarnath Natarajan, Ronald Veselenak, Nigel Bourne, Scott Gilbertson

University of Texas Medical Branch, Galveston, TX

Background: Central serotonin (5-HT)_{2C} receptors (5-HT_{2C}R) in prefrontal cortical regions play an important role in psychological disorders marked by impulsive-compulsive traits (e.g., addiction, depression, eating disorders), and strategies to augment 5-HT_{2C}R signaling may prove therapeutically useful in such disorders. Studies indicate that a protein complex forms between the 3rd intracellular loop of the 5-HT_{2C}R and *protein phosphatase and tensin homologue deleted on chromosome 10* (PTEN), a tumor suppressor and participant in brain development. The goal of the present study was to detail the structural and functional significance of the 5-HT_{2C}R:PTEN interaction and to evaluate the biological impact of disruption of the protein complex with a small peptide fragment (15 amino acids) of the 5-HT_{2C}R (3L4F; Pro283-Arg297), which competes with the 5-HT_{2C}R for binding to PTEN.

Methods: Co-immunoprecipitation studies for the 5-HT_{2C}R and PTEN were conducted in rat prefrontal cortex synaptosomes as well as Chinese Hamster Ovary (CHO) cells stably transfected with the 5-HT_{2C}R (5-HT_{2C}R-CHO cells) to determine association of the two proteins within a complex. The 5-HT_{2C}R function was measured in 5-HT_{2C}R-CHO cells using intracellular calcium efflux as a readout. To further validate that the 5-HT_{2C}R and PTEN proteins are in *direct* contact, we developed a novel split luciferase complementation assay (LCA) in live cells. In this reporter system, two complementary N-(NLuc) and C-terminus (CLuc) components of the luciferase enzyme, which have no activity on their own, were fused to the 15 amino acids of 3rd intracellular loop of the 5-HT_{2C}R (i.e., the domain which binds PTEN) and PTEN, respectively. In the presence of the substrate D-luciferin, association of the 5-HT_{2C}R and PTEN brings the inactive luciferase fragments into close proximity and the enzyme activity is reconstituted.

Results: Co-immunoprecipitation studies in rat prefrontal cortex synaptosomes and 5-HT_{2C}R-CHO cells confirmed that the 5-HT_{2C}R and PTEN are part of a protein complex *ex vivo* and *in vitro*. The 3L4F peptide disrupted this complex in the 5-HT_{2C}R-CHO cells in a time-dependent manner and pretreatment with 3L4F enhanced 5-HT (10 nM) stimulated intracellular calcium efflux in a concentration-related manner ($EC_{50} \approx 7$ nM), suggesting that the 5-HT_{2C}R:PTEN complex is essential to the efficiency of the 5-HT_{2C}R signalingosome. Furthermore, the LCA detected a *direct* interaction between the two proteins, and preliminary studies suggest that the 3L4F peptide decreases production of the luminescence signal emitted upon formation of the 5-HT_{2C}R:PTEN complex.

Discussion: These data suggest that molecules that inhibit the 5-HT_{2C}R:PTEN association will be novel pharmacological means to enhance 5-HT_{2C}R function. To further the goal to identify small molecules with this profile, additional studies are underway to optimize the LCA assay as a high throughput screen to detect peptides/molecules based on the 3L4F scaffold that disrupt this protein complex as a means to identify compounds that may prove therapeutically promising in disorders in which disrupted 5-HT_{2C}R signaling is implicated.

Disclosure: K. Cunningham: Part 1; Wyeth. F. Laezza: None. A. McGinnis: None. P. Seitz: None. A. Shavkunov: None. A. Agarkov: None. N. Panova-Elektronova: None. N. Anastasio: None. L. Fink: None. C. Watson: None. A. Natarajan: None. R. Veselenak: None. N. Bourne: None. S. Gilbertson: None.

32. Homology Modeling of ABC Transporters and Neurotransmitter Transporters

Aina W. Ravna, Mari Gabrielsen, Kurt Kristiansen, Ingebrig Sylte, Georg B. Sager, Svein G. Dahl*

University of Tromsø, Tromsø Norway

Background: Molecular models of transporter proteins may be constructed by homology modeling using as a template a transporter protein with known three-dimensional (3D) crystal structure and a certain sequence similarity (homology). In constructing homology models of transporters, there are several pitfalls in the procedure. The few available experimental template structures generally have a low resolution, and the homology between the modeled transporter and the template may also be low. A sequence identity below 30% may be considered “borderline” of what can be considered as a basis for constructing realistic models. Structure-based drug design based on low-homology models may not be as applicable as design based on models with sequence identities above ~50%. Even when the sequence homology is low, the 3D structures of homologous proteins are generally more conserved than their sequence. Low-homology models may therefore be useful for assignment of protein folding and function. Such models also provide tools for suggesting candidate residues for mutagenesis experiments, and active sites may be identified by combined molecular modeling and site directed mutagenesis studies.

Methods: Here we present molecular models of ABC transporters and neurotransmitter transporters based on various templates. We have constructed outward-facing molecular models of ABCB1 (P-glycoprotein), ABCC4 and ABCC5 based on the *Staphylococcus aureus* ABC transporter Sav1866, which has been crystallized in an outward-facing ATP-bound state, and inward facing models of ABCB1, ABCC4 and ABCC5 based on a wide open inward-facing conformation of *Escherichia coli* MsbA.

Results: After the models were constructed, our methodology was validated when the X-ray crystal structure of the *Mus musculus* ABCB1 in a drug-bound conformation was reported. Amino acids forming a putative substrate recognition site in the ABCB1 models were confirmed by the ABCB1 X-ray crystal structure. We have also constructed models of the dopamine transporter (DAT), the serotonin transporter (SERT), and the noradrenalin transporter (NET) based on *Aquifex aeolicus* LeuTAA crystal structures in substrate-bound and inhibitor-bound conformations, and compared the models with site directed mutagenesis data.

Discussion: These transporter models are examples of how structural information and insight can be obtained even from models based on low-homology and low-resolution templates. Transporters may undergo substantial conformational changes during the transport cycle, and when interpreting homology models of transporters and performing docking studies on such models, the structural flexibility of transporters should be considered. These models should be regarded as working tools for generating hypotheses and designing further experimental studies related to ABC transporter and neurotransmitter structure and function. The possible limitations of these models due to

a limited sequence similarity and structural resolution of the applied template should be kept in mind.

Disclosure: A. Ravna: None. M. Gabrielsen: None. K. Kristiansen: None. I. Sylte: None. G. Sager: None. S. Dahl: None.

33. Role of Phosphodiesterase 4 Variants in Depression

Yogesh Dwivedi*, Hui Zhang, Ghanshyam Pandey, Daniel Dlaboga

University of Illinois at Chicago, Chicago, IL

Background: cAMP-specific phosphodiesterases (PDE)4 are a family of enzymes capable of hydrolyzing cAMP to 5'AMP. cAMP plays a major role in synaptic plasticity, neurite outgrowth, and neuronal differentiation and survival and recent studies demonstrate involvement of cAMP in pathophysiology of depression. The cAMP-specific PDE4 can be differentiated from other PDE families by sequence identity in the catalytic region of the protein and by their ability to be inhibited by a specific class of drugs, of which rolipram is the prototype. PDE4 enzymes are also unique in having 'signature' regions of sequence, called upstream conserved regions (UCR1 and UCR2), located in the N-terminal region of the proteins. There are four PDE4 subfamilies, encoded by separate genes (PDE4A, PDE4B, PDE4C, PDE4D), all are expressed in brain. Although, several studies suggest that PDE inhibitors show antidepressant-like effects in rodents, the role of PDEs in depression is not known. To examine the role of PDE4 in depression, we characterized various isoforms of PDE4 in human brain and examined whether there are alterations in specific PDE4 variant(s) in depressed brain.

Methods: Expression of PDE4A1, PDE4A4, PDE4A8, PDE4A10, PDE4A11, PDE4B1, PDE4B2, PDE4B3, PDE4D3, PDE4B5, PDE4D1, PDE4D4, PDE4D5, PDE4D6, PDE4D7, and PDE4D9 variants were characterized by qPCR in prefrontal cortex and hippocampus obtained from normal control subjects. In addition, subcellular distribution of certain PDE4 variants was characterized using specific antibodies. In addition, we compared PDE4 catalytic activity and expression of PDE4 variants between normal controls and depressed suicide subjects.

Results: We found that expression of PDE4A4, PDE4A10, PDE4B3, PDE4D4, PDE4D6, and PDE4D7 variants were highly expressed in human brain. On the other hand, PDE4D8 was not expressed in human brain. Subcellular distribution studies suggest that PDE4A1, PDE4A4, and PDE4D9 were absent in cytosol fraction, whereas PDE4D3 was absent in membrane fraction. When compared, we found that catalytic activity of PDE4 and expression levels of PDE4A1, DE4B2, and PDE4A4 were significantly decreased in PFC and hippocampus of depressed suicide subjects.

Discussion: Our study for the first time provides evidence of variant specific alterations in PDE4 in brain of depressed subjects and indicates that these variants may be used as site-specific therapeutic target(s) to develop novel antidepressants.

Disclosure: Y. Dwivedi: None. H. Zhang: None. G. Pandey: None. D. Dlaboga: None.

34. Amiodarone's Interactions With Muscarinic Receptors: New Concepts In Allosteric Regulation Of Receptor Function

John Ellis*, Edward Stahl, Gwendolynne Elmslie

Penn State College of Medicine, Hershey, PA

Background: The concept of allosteric regulation of receptor function has come to be appreciated more and more in recent years. Briefly, some of the potential advantages are: allosteric sites can allow greater selectivity across subtypes of a receptor family, because they are typically less well conserved than their orthosteric counterparts; there can be a decreased danger of over-dose toxicity, because allosteric ligands exhibit ceiling effects; and, especially important to CNS synaptic systems, allosteric regulation is uniquely suited to preserving spatio-temporal patterning of receptor activation. Allosteric sites on muscarinic acetylcholine receptors have been extensively studied, both for potential therapeutic benefits and as a model system for the Class A family of

G-protein coupled receptors. We report here on the nature of amiodarone's interactions with a novel allosteric site on muscarinic receptors.

Methods: Receptors were expressed in CHO cells. Binding assays were carried out with radiolabeled N-methylscopolamine (NMS), in membranes derived from those cells, using atropine to define nonspecific binding. Responses of Gq-coupled receptors were measured in intact cells. The release of arachidonic acid was measured by the BSA-binding technique and the accumulation of inositol monophosphate in the presence of lithium was measured using Dowex-formate columns. Curvefitting and simulations to empirical and mechanistic equations was accomplished using GraphPad Prism. **Results:** Binding assays found that amiodarone was able to inhibit the binding of radiolabeled NMS at all subtypes, but that this inhibition was incomplete. Furthermore, the rate of dissociation of NMS from the receptors was slowed in the presence of amiodarone. In response assays, amiodarone enhanced the maximal level of agonist-stimulated response at M₁, M₃, and M₅ receptors, with variable effects on potency. That is, agonist potency was enhanced at M₃, unchanged at M₅, and reduced at M₁. N-ethylamiodarone (NEA) did not alter maximal agonist response and only reduced agonist potency (that is, it appeared to be an antagonist). However, the action of NEA could be clearly distinguished from the action of the orthosteric antagonist NMS. When an agonist concentration-response curve was generated in the presence of amiodarone, the addition of NMS shifted the curve to the right; however, amiodarone's enhancement of maximal response was preserved. By contrast, when NEA was added to the same agonist concentration-response curve (in the presence of amiodarone), the curve was shifted to the right and amiodarone's enhancement of maximal response was eliminated. These results are consistent with NMS being competitive with the orthosteric agonist, and NEA being competitive with amiodarone at their novel allosteric site (see Discussion, below).

Discussion: Both the binding studies and the functional studies confirm that amiodarone must be acting allosterically. Additional binding studies are conclusive that amiodarone does not act at the "common" allosteric site that we have defined previously. Functional studies of the interaction between amiodarone and NEA are entirely consistent with the model that these two ligands are competitive at the novel allosteric site. Demonstration of this point is facilitated by a new elaboration of Hall's allosteric two-state model that we have developed; this model represents a system composed of two ligands that compete with each other at the orthosteric site (e.g., an agonist and the competitive antagonist NMS) and two ligands that compete with each other at the allosteric site (e.g., amiodarone and NEA). Also, the finding that amiodarone is competitive with the positively charged analog NEA indicates that the allosteric site lies in the extracellular portion of the receptor. Finally, we are able to demonstrate a new and unique form of allosteric modulation, which we term signal sharpening and which depends upon the relationship between the values of two of the parameters of Hall's original model. This concept of signal sharpening is predicted theoretically by the model and is experimentally observed in studies of amiodarone's actions at the M₁ receptor. Sharpening occurs in the sense that responses above a certain magnitude are enhanced, while responses below that magnitude are reduced. This suggests the possibility that drugs could be designed to enhance "true" synaptic signaling events, while suppressing weaker "false" signals (e.g., signals that are produced by transmitter leakage or by spillover from nearby synapses).

Disclosure: J. Ellis: None. E. Stahl: None. G. Elmslie: None.

35. Neuronal Progenitor Cells Derived from Bipolar Patients are More Susceptible to Apoptosis than Cells Obtained from Unaffected Controls

Rif El-Mallakh*, Yonglin Gao, Zhenmin Lei, Welby Winstead, C Lu, Fred Roisen

University of Louisville School of Medicine, Louisville, KY

Background: The course of bipolar disorder is characterized by reduced brain volume as a function of recurrence. This tissue loss is

believed to be due to apoptosis. Examination of apoptosis can be accomplished in a cellular model of the illness.

Methods: Olfactory neuroepithelial neuronal progenitors have been established from biopsies performed on bipolar I subjects and unaffected controls. These cells have the genetic heritage of the illness, are immortal in culture, and spontaneously differentiate into neurons and glia. Cells were treated with monensin 10^{-6} M for 24 hours. Monensin is a sodium ionophore that induces a dose- and time-related increase of intracellular sodium (Na) and calcium (Ca). Apoptosis was measured by quantification of measured histones H1, H2A, H2B, H3, and H4. The cytoplasmic fraction was isolated from 1×10^5 cells/sample used the antigen was the source in a sandwich ELISA.

Results: Apoptosis was greater in cells obtained from bipolar subjects compared to non-bipolar subjects ($P = 0.02$).

Discussion: Bipolar illness may be associated with increased susceptibility to pro-apoptotic stimuli. If confirmed, this would have major implications regarding the pathophysiology of bipolar disorder and would highlight the importance of using neuroprotective treatments.

Disclosure: R. El-Mallakh: Part 1; Honararia: AstraZeneca, Bristol-Myers Squibb, Lilly, Pfizer, Merck. Y. Gao: None. Z. Lei: None. W. Winstead: None. C. Lu: None. F. Roisen: Rhinocyte. Part 2; Rhinocyte.

36. Fragile X Mental Retardation Protein Is Reduced in Cerebellar Vermis and Superior Frontal Cortex of Subjects with Autism

S. Hossein Fatemi*, Timothy D. Folsom, Rachel E. Kneeland, Stephanie B. Liesch

University of Minnesota, Minneapolis, MN

Background: Fragile X syndrome (FXS) has multiple phenotypes in common with autism including brain structural abnormalities, presence of seizures and cognitive and behavioral deficits. FXS is caused by loss of function of the fragile X mental retardation-1 gene (FMR1) and subsequent loss of its product, fragile X mental retardation protein (FMRP). Because of the overlap in phenotypes, we investigated whether FMRP is similarly reduced in subjects with autism.

Methods: Postmortem samples were derived from subjects with autism ($N = 16$ for cerebellar vermis and $N = 20$ for superior frontal cortex (BA9)) and matched controls ($N = 11$ for cerebellar vermis and $N = 10$ for BA9). SDS-PAGE and western blotting were used to measure protein levels. We measured protein levels of FMRP, metabotropic glutamate receptor 5 (mGluR5), GABA_A receptor beta 3 (GABRB3). All protein measurements for subjects with autism and control subjects were normalized against β-actin and neuronal specific enolase (NSE). Group differences were analyzed statistically using student's t-test. Significant differences were defined as those with a p value < 0.05 . Confound effects (i.e., age and PMI) were examined using Pearson's correlation test. Additionally, qRT-PCR was used in BA9 tissue to measure mRNA levels of FMR1, GABRB3, GABRD, GRM1, and GRM5. **Results:** We demonstrated significant reduction in levels of FMRP in the vermis of adults with autism ($p < 0.028$) when compared with age and PMI matched controls. Additionally, we observed that levels of mGluR5 protein is significantly increased in vermis of children with autism ($p < 0.0023$ for dimer and $p < 0.042$ for total mGluR5). We have also demonstrated that the expression of mGluR5 in autistic children is significantly more homo-dimerized ($p < 0.049$) when compared with normal children. There was also a significant decrease in GABRB3 in vermis of adult subjects with autism ($p < 0.031$). In BA9 of subjects with autism we similarly found reduced expression of FMRP in adults ($p < 0.017$) and increased mGluR5 in children ($p < 0.013$ for dimer and $p < 0.04$ for total mGluR5). Moreover, in BA9 qRT-PCR found a reduction in FMR1 mRNA ($p < 0.026$).

Discussion: Our results are the first to demonstrate reductions in FMRP in two brain regions from subjects with autism when compared with matched controls. Reduced levels of FMRP and increased levels of mGluR5 may contribute to abnormalities that are common between autism and FXS. Grant support by NICHHD (5R01-HD052074-04 and 3R01-HD052074-03S1) is appreciated.

Disclosure: S. Fatemi: None. T. Folsom: None. R. Kneeland: None. S. Liesch: None.

37. Elucidating the Role of Striatal Pathways in Operant Responding and Decision-Making Tasks Using Targeted Viral-Mediated Gene Transfer of DREADD Receptors in Rats

Susan Ferguson*, Daniel Eskanazi, Paul Phillips, John Neumaier

University of Washington, Seattle, WA

Background: A key feature of many neuropsychiatric disorders, such as drug addiction and obsessive-compulsive disorder, is the display of aberrant reinforcement learning and decision making processes. Dysregulation of the striatum is thought to contribute to these disorders; however, the striatum is a heterogeneous structure containing distinct populations of GABAergic medium spiny projection neurons (MSNs) that differ in their neuropeptide composition and form two major efferent pathways. MSNs that contain the neuropeptides dynorphin and substance P are part of the striatonigral, or 'direct' pathway whereas MSNs that contain the neuropeptide enkephalin are part of the striatopallidal, or 'indirect' pathway. The role of these specific striatal sub-types in reinforcement learning and decision-making is not yet known.

Methods: As a first step toward addressing this issue, we used a novel chemical-genetic approach to determine how decreasing activity of the striatonigral (or striatopallidal) pathway would alter performance of operant learning and decision-making tasks. Briefly, we developed viral vectors that use the preprodynorphin (or preproenkephalin) promoter to target expression of either green fluorescent protein or hemagglutinin-tagged Gi/o-coupled human M4 DREADD (designer receptor exclusively activated by a designer drug) receptors to striatonigral (or striatopallidal) neurons; the latter allows for transient reduction of neuronal excitability using the pharmacologically inert synthetic ligand clozapine-N-oxide (CNO).

Results: After viral infusion and DREADD receptor expression into the dorsomedial striatum of Long Evans rats, we found that transiently decreasing activity of striatonigral neurons had no effect on the performance of a decision making task for small versus large magnitude natural rewards (i.e., 1 or 4 food pellets). However, decreasing activity of these neurons during a simple operant task disrupted the acquisition of lever pressing for a sugar reward. Studies are currently underway to determine whether acquisition of a decision making task is similarly impaired.

Discussion: By using techniques that allow for the selective disruption of neuronal activity *in vivo*, these studies should allow us to help elucidate the contribution of specific striatal cell populations in the acquisition and maintenance of operant responding and decision-making tasks.

Disclosure: S. Ferguson: None. D. Eskanazi: None. P. Phillips: None. J. Neumaier: None.

38. Randomized, Placebo-Controlled Study Of Levothyroxine As Add-on Treatment In Bipolar Depression: Higher Efficacy In Women Than In Men

Michael Bauer*, Mazda Adli, Ursula Köberle, Maximilian Pilhatsch, Cathrin Sauer, Peter C. Whybrow, Mark Frye, Thomas Stamm

University Hospital Carl Gustav Carus, Dresden, Germany

Background: Thyroid axis dysfunction may contribute to the pathophysiology of bipolar illness. Evidence emerged from acute and long-term intervention studies that add-on treatment with supra-physiological doses of levothyroxine (L-T4) may be an effective medication to augment and stabilize patients with bipolar disorder.

Methods: Multicenter, 6-week, double-blind, randomized, parallel-group, placebo-controlled fixed-dose trial assessing efficacy and tolerability of (L-T4) (300 mcg/d) adjunctive to continuing treatment

with mood stabilizer and/or antidepressant medication for patients with bipolar disorder (type 1 and 2; both gender, 18 years or older) currently in a major depressive episode (DSM-IV). A 1-week, single-blind placebo run-in-phase was added to screen potential patients for study inclusion. Other inclusion criteria: prior randomization stable dosage of mood stabilizer and/or antidepressant for at least 2 weeks, and for at least 2 weeks, serum levels of mood stabilizer within therapeutic ranges, antidepressants at standard doses. Most relevant exclusion criteria: ultra-rapid cyclers, abnormal basal TSH levels, thyroid hormone medication within 4 weeks before screening. Ongoing mood stabilizer/antidepressant treatment was maintained throughout the study, allowing minor changes in the doses if necessary for tolerability issues. Primary endpoint was change from randomization at week 6 in HAMD total score; secondary efficacy variables included change in MADRS total score. Adverse events were reported throughout the study measured using the Thyroid Symptom List (TSL). Efficacy analyses were conducted in the intent-to-treat (ITT) population (patients who received at least 1 dose of study medication and had at least 1 post-baseline efficacy assessment) using last observation carried forward (LOCF) methodology. Analysis of variance (ANOVA) models were used to assess the difference between the groups in outcome measures at study end with age, sex and baseline values as covariates.

Results: Of 75 patients enrolled in the study, 63 (32 female; mean age 45.1 years) were randomized. Twelve patients were excluded due to the following reasons: screening failure (two patients with overt thyroid disease), withdrawal of consent, early placebo response during the single-blind run-in-phase or missing HAMD at randomisation. Twelve patients dropped out of the study during double-blind treatment because of lack of response, switch to mania, lost to follow-up and adverse events. There were no statistically differences between groups in demographic and clinical characteristics. Mean HAMD score at the time of randomization was 20.9 ± 3.0 in the L-T4 group, 21.4 ± 4.3 in the placebo group. Mean change in HAMD score from randomization to week 6 was larger in the L-T4 compared to placebo group (-7.9 (38.7%) vs. -5.1 (25.5%)). The difference between groups adjusted for age, sex and HAMD score at randomization did not reach statistical significance. The course of HAMD scores over time from randomization to week 6 (end of double-blind treatment) adjusted for age was not significantly different between groups ($p = 0.438$). The proportion of patients who experienced response ($\geq 50\%$ reduction in HAMD score) at week 6 was 38% for L-T4 and 26% for placebo; the difference between groups was statistically not significant ($p = 0.319$). Remission rates at week 6 were 22% for L-T4 and 16% for placebo ($p = 0.561$). However, in women (placebo N=15, L-T4 N=17) there was a significant difference between groups in mean change of HAMD score (-16.6% placebo vs. -42.4% L-T4, $p = 0.018$). When adjusted for age, the latter effect was not significant but showed a clear trend ($p = 0.073$). Overall tolerability of L-T4 was good; no serious adverse event occurred. TSL sum score, blood pressure and body weight did not differ significantly between both study groups in the beginning and the end of the study.

Discussion: This is the first study to evaluate efficacy and tolerability of supraphysiological doses of L-T4 in bipolar depressed patients in a placebo-controlled study. Patients treated with L-T4 did numerically better than those treated with placebo, however, the study failed to detect a statistically significant difference between the two groups. A better improvement in depression scores with L-T4 was detected in women but not in men. Acknowledgment: The study was sponsored by the Stanley Medical Research Institute (grant o2T-238 to M.B.).

Disclosure: M. Bauer: Part 1; AstraZeneca, Lilly, Servier, Janssen-Cilag, Lundbeck, BMS, Otsuka, GlaxoSmithKline, Pfizer. M. Adli: AstraZeneca, Lilly, GlaxoSmithKline, Pfizer, Boehringer Ingelheim, Sanofi-Aventis, Esparma, Wyeth. Part 4; Esparma, Lundbeck. U. Köberle: None. M. Pilhatsch: None. C. Sauer: None. P. Whybrow: None. M. Frye: Part 1; Astra-Zeneca, Bristol-Myers Squibb, Cephalon, Lilly, GlaxoSmithKline, Merck, Otsuka Pharmaceuticals, Pfizer,

Sanofi-Aventis, Dainippon Sumitomo Pharma, Janssen Pharmaceuticals, Johnson & Johnson, Pfi. Part 4; Pfizer, National Alliance for Schizophrenia and Depression (NARSAD), Mayo Foundation. T. Stamm: None.

39. Use of Targeted Proteomics to Identify Novel Abeta Isoforms in Cerebrospinal Fluid as Biomarkers for Gamma-Secretase Inhibitor Treatment in Alzheimer's Disease

Kaj Blennow*, Henrik Zetterberg, Erik Portelius

Clinical Neurochemistry Lab, Mölndal, Sweden

Background: Intense multi-disciplinary research efforts have provided detailed knowledge on the molecular pathogenesis of Alzheimer's disease (AD). The prevailing hypothesis for AD is the amyloid cascade hypothesis, which states that beta-amyloid (Abeta) aggregation with plaque formation is the central event, ultimately leading to neuronal degeneration and clinical symptoms. There are a large number of drug candidates that are now in clinical trials that may have disease-modifying effects. The vast majority of these aims to inhibit Abeta toxicity, and include for example secretase inhibitors, which inhibit the production of Abeta from APP. The effect of disease-modifying anti-Abeta drugs on plaque pathology is commonly evaluated in AD transgenic mice; however, these animal models have a low predictive power for treatment success in patients with sporadic AD. To bridge the gap between animal studies and large clinical trials, biomarker evidence from small scale trials that a drug has a true effect on the AD disease process directly in man would help in selecting the most promising drug candidates for large and expensive Phase 2 and 3 clinical trials.

Methods: We used the targeted proteomics approach to search for APP and Abeta isoforms that could serve as biomarkers in clinical trials. We developed a novel technique based on immunoprecipitation (IP) with an anti-Abeta monoclonal antibody combined with matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry, to identify and quantify all APP and Abeta isoforms simultaneously. Novel isoforms were identified by tandem MS/MS on a hybrid linear ion trap Fourier transform ion cyclotron resonance (LTQ FT-ICR) mass spectrometry instrument.

Results: In addition to Abeta1-42 and Abeta1-40, we were able to identify a series of shorter carboxy-terminally truncated Abeta isoforms. In experimental studies on APP transfected cells, we showed that the short Abeta isoforms Abeta1-14, Abeta1-15 and Abeta1-16 are produced by a novel pathway of APP processing involving the concerted actions of beta-secretase and alpha-secretase, while the longer isoforms (Abeta1-17 up to Abeta1-42) are produced by the gamma-secretase pathway. In experimental studies in AD transgenic mice we found that gamma-secretase inhibitor treatment results in a marked increase in Abeta1-14, 1-15 and 1-16, probably due to an increase substrate availability of the C99 APP stub (APP beta-CTF) induced by gamma-secretase inhibition. This finding could also be verified in cerebrospinal fluid (CSF) samples from dogs treated with a gamma-secretase inhibitor (LY450139). Last, in a phase II clinical trial including AD patients, the CSF levels of Abeta1-14/15/16 increased dose-dependently in response to treatment with the gamma-secretase inhibitor LY450139. In the same trial, there was only trend for a decrease in the longer isoforms Abeta40 and Abeta42.

Discussion: We conclude that targeted proteomics is a valuable technique in the search for novel biomarkers for brain disorders such as AD. The newly identified short Abeta isoforms are generated through a previously unrecognized metabolic pathway for APP processing. These novel Abeta isoforms may also serve as sensitive biomarkers in AD drug development.

Disclosure: K. Blennow: Part 1; Innogenetics, AstraZeneca, Pfizer, Merz Pharmaceuticals, Bristol-Myers Squibb, Eisai, Janssen Immunotherapy, Sanofi Avensis. Part 4; Bristol-Meyers Squibb, USA, Innogenetics, Ghent, Belgium. H. Zetterberg: None. E. Portelius: None.

40. Omega-3 Fatty Acids for the Treatment of Depression: Systematic Review and Meta-Analysis

Michael Bloch*, Jonas Hannestad

Yale University, New Haven, CT

Background: Pharmacological and behavioral treatments for Major Depressive Disorder (MDD) show only modest efficacy. Evidence from both ecologic, cross-sectional and case-control studies suggest that fish consumption and omega-3 fatty acid (FA) intake may affect the prevalence of MDD. Because omega-3 FAs are anti-inflammatory and are preferentially incorporated in the brain, a high dietary omega-6 to omega-3 FA ratio could promote neuroinflammation. Increased omega-3 FA concentration in the diet may also act by altering CNS cell membrane fluidity and phospholipid composition which may alter the structure and function of the proteins embedded in it. By this mechanism, increased omega-3 FA concentrations in cell membranes have been shown to affect serotonin and dopamine neurotransmission. Several but not all clinical trials of omega-3 FAs in treatment of MDD have suggested efficacy. Previous meta-analyses have demonstrated a statistically significant beneficial effect of omega-3 FAs in the treatment of MDD but failed to account for potential publication bias in the literature.

Methods: We conducted a meta-analysis of randomized, placebo controlled trials of omega-3 fatty acid treatment of unipolar depression in order to determine efficacy and to examine sources of heterogeneity between trials. PubMED was searched for randomized, placebo-controlled trials of omega-3 fatty acids in the treatment of unipolar depression. Our primary outcome measure was standardized mean difference in a clinical measure of depression severity. In stratified meta-analysis we examined the effects of trial duration, baseline depression severity, diagnostic indication, dose of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in omega-3 preparations and whether omega-3 fatty acid was given as monotherapy or as augmentation of traditional antidepressants.

Results: In 13 randomized, placebo-controlled trials examining the efficacy of omega-3 fatty acids involving 731 participants, meta-analysis demonstrated no significant benefit of omega-3 fatty acid treatment compared to placebo ($SMD = 0.11$, 95% CI: -0.04 , 0.26). Meta-analysis demonstrated significant heterogeneity and publication bias. Nearly all evidence of omega-3 benefit was removed after adjusting for publication bias using the trim-and-fill method ($SMD = 0.01$, 95% CI: -0.13 , 0.15). Stratified analysis suggested increased efficacy of omega-3 fatty acid treatments when higher doses of EPA were used and in trials where study participants had greater baseline depression severity.

Discussion: Current published trials suggest a small, non-significant benefit of omega-3 fatty acids for unipolar depression. Most of the treatment efficacy observed in the published literature may be attributable to publication bias.

Disclosure: M. Bloch: None. J. Hannestad: None.

41. Dose Analyses and Relapse Characterization from a Bipolar Maintenance Study of Ziprasidone Adjunctive to Lithium or Valproate

Charles Bowden*, Onur Karayal, Jeff Schwartz, Balarama Gundapaneni, Manuel Garcia, Cedric O'Gorman

University of Texas Health Science San Antonio, San Antonio, TX

Background: In clinical studies, ziprasidone has been shown to be an effective monotherapy for acute bipolar disorder, manic or mixed episodes and more recently an adjunctive treatment to either lithium or valproate in the maintenance treatment of bipolar I disorder. The probability of remaining relapse-free on adjunctive ziprasidone was consistently higher across 6 months than on either lithium or valproate alone. The objectives of these post-hoc analyses were to look at relapse prevention by dose and to characterize the relapses observed.

Methods: Study A1281137 was a Phase 3, placebo-controlled outpatient trial evaluating the maintenance of effect of ziprasidone plus lithium or

valproate in non-stabilized subjects (MRS ≥ 14 despite therapeutic levels of or lithium or valproate) with a recent or current manic or mixed episode of bipolar I disorder. The trial comprised a 2.5-4 month, open-label period during which patients were stabilized on adjunctive ziprasidone followed by a 6 month, double-blind maintenance period (data reported here). Stabilization was defined as a CGI-I ≤ 3 for 8 weeks with stable doses of all medications for at least the last 4 weeks. Patients were stabilized on adjunctive ziprasidone dosed at 80, 120 or 160 mg/day given bid. Upon randomization, subjects either continued on the same fixed dose or ziprasidone was tapered and replaced with placebo. The primary endpoint was time to intervention for a mood episode, and the key secondary endpoint was time to discontinuation for any reason. In both cases, adjunctive ziprasidone was statistically superior to lithium or valproate alone. Here we analyze these two endpoints (Kaplan-Meier survival analysis) by dose versus placebo and further characterize the type and timing of relapses observed.

Results: Analyses by dose revealed that the probability of being relapse-free versus placebo for the 80 mg, 120 mg and 160 mg dose groups was associated with a p-value of 0.162, 0.004 and 0.395 respectively. Of patients who were stabilized and randomized on the 80 mg, 120 mg and 160 mg doses of adjunctive ziprasidone, 23% (14/60), 10% (4/40) and 26% (7/27) respectively relapsed. The probability of continuing treatment versus placebo for the 80 mg, 120 mg and 160 mg dose arms was associated with a p-value of 0.2, 0.001 and 0.2 respectively. Of patients who were stabilized and randomized on the 80 mg, 120 mg and 160 mg doses of adjunctive ziprasidone respectively, 42% (25/60), 20% (8/40) and 37% (10/27) discontinued for any reason. Depressive, manic, and mixed episodes accounted for 53%, 34%, and 13%, respectively, of the total number of 61 relapse events in the study. 9/127 on ziprasidone and 20/111 on placebo relapsed into manic/mixed while 16/127 on ziprasidone and 16/111 on placebo relapsed into depression. By the end of the first 4 weeks, almost half (48% [12/25]) of the relapses seen in the ziprasidone group and almost two-thirds (64% [23/36]) of those seen in the placebo group had occurred. By week 8 these rates rose to 64% and 72% respectively. Kaplan Meier curves reflecting time to relapse (overall, manic/mixed and depressive) show that depressive relapses tended to occur earlier with 75% (24/32) having occurred by the end of week 4. Manic/mixed relapses were more smoothly distributed over time.

Discussion: These analyses further characterize relapse prevention seen with adjunctive ziprasidone in terms of dosing and further describe the type and timing of relapse observed. Almost half of patients who were stabilized in the open-label phase received the 80 mg dose while the relapse-prevention effect within the double-blind phase was largely driven by the 120 mg dose. These results suggest that a dosage of 120 mg/day of ziprasidone will yield better adjunctive maintenance effectiveness than lower or higher doses for most bipolar I patients. Most relapses were depressive in nature and the majority of total relapses occurred within the first 8 weeks of the 24 week double-blind period.

Disclosure: C. Bowden: Part 1; BMS, Sanofi Aventis, Schering, Pfizer. Part 4; Abbott, GSK, Repligen, Janssen. O. Karayal: Part 5; Pfizer. J. Schwartz: Pfizer. B. Gundapaneni: Pfizer. M. Garcia: Pfizer. C. O'Gorman: Pfizer.

42. Ziprasidone D₂ Receptor Occupancy at Doses of 120 to 240 mg/day

Measured with ¹⁸F-Fallypride PET Support Once-A-Day Dosing

William Bunney*, David Keator, James Fallon, Marilyn Kesler-West, Adrian Preda, Dana Nguyen, Jogesh Mukherjee, Nikunj Shah, Steven Potkin

University of California Irvine, School of Medicine, Irvine, CA

Background: Ziprasidone is an atypical antipsychotic medication approved to treat schizophrenia and bipolar disorder dosed twice per day in a range of 80 to 160 mg/day. Ziprasidone has a plasma half-life

of 5 to 7 hours suggesting that multiple dosing might be required to obtain sufficient D₂DR occupancy consistent with clinical efficacy. Previous studies have suggested than approximately 65% D₂ receptor occupancy is needed to achieve antipsychotic efficacy. However, the duration of D₂DR occupancy and regional requirements for occupancy have not been well studied. Previous ziprasidone studies have found a relationship between striatal D₂DR occupancy and plasma ziprasidone concentrations but not with dose. These studies also did not investigate doses above 160 mg/day. To more fully explore the time course of D₂DR we conducted a prospective study of schizophrenia people on steady state doses of 120 to 240 mg/day p.o. ziprasidone. We present D₂DR occupancy results.

Methods: Thirteen male and female people with schizophrenia meeting DSM-IV criteria were randomly assigned to monotherapy with either 60 mg bid, 80 mg bid, or 120 mg bid, for greater than 2 weeks. ¹⁸F-fallypride PET scans with a high resolution HRRT camera were obtained at three possible time points: 5 hours, 12 hours or 25 hours post the last administered dose of ziprasidone. Each subject was randomly assigned to have PET scans at two of these time points to create a 24 h time activity curve. Ten subjects completed the study. The ¹⁸F-fallypride PET scan for D₂ receptor occupancy was obtained immediately after IV injection of 6.0 ± 0.9 SD mCi as a bolus over one minute. Emission scans were obtained at 6 frames of 30 s, 7 frames of 60 s, 5 frames of 120 s, 4 frames of 300 s, and 4 frames of 600 s followed by a short transmission scan for attenuation and scatter correction. The subjects then had a 20 minute break and returned to the scanner for emission scans acquired at 8 frames of 600 s followed by a second transmission scan. Each subject received a MRI that was segmented with Freesurfer and then transferred to the PET scans for coregistration. D₂DR occupancy for each region of interest (ROIs) contrasted with the cerebellum were calculated and compared to occupancy in the cerebellum an area with low D₂DR and contrasted with previously collected D₂DR data sets in antipsychotic-free healthy controls (J. Mukherjee, et al. Synapse 46:170-188 (2002)). Distributed volume ratio (DVR) estimates were obtained graphically from PET scans without blood sampling using the reference region method described by Logan et al. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. 1996. Distribution volume ratios without blood sampling from graphical analysis and PET data. J Cereb Blood Flow Metab 16:834-840.) The primary endpoints, were occupancy in the caudate and putamen at various time points. Occupancy in nucleus accumbens and nonstriatal ROIs including the thalamus, and amygdala were determined.

Results: D₂DR occupancy at 5 hours after last dose was $83 \pm 8.8\%$ SD; at 12 hours was $80 \pm 10.6\%$ SD; and at 24 hours $71 \pm 20.7\%$ SD in caudate. Occupancies in the putamen were very similar to those in the caudate: 81%, 78% and 69% as were those in the thalamus and amygdala. Occupancies at corresponding time points in the nucleus accumbens were slightly lower than other areas measures: 74%, 70% and 59%. No occupancy dose relationship was observed.

Discussion: These data indicate that on average high levels of D₂DR occupancy are maintained 24 hours after the last dose of ziprasidone supporting the common clinical practice of QD dosing for many patients. In addition, these data suggest that some patients require 240 mg/day to achieve the levels of D₂DR occupancy associated with clinical response.

Disclosure: W. Bunney: None. D. Keator: None. J. Fallon: None. M. Kesler-West: None. A. Preda: None. D. Nguyen: None. J. Mukherjee: None. N. Shah: None. S. Potkin: Part 1; Arbor Scientia, AstraZeneca, Bioline, Bristol-Myers Squibb, Elan, Lilly, Janssen Pharmaceutica, Merck, Novartis, Otsuka, Pfizer, Sepracor Dainippon-Sumitomo, U of So.Calif, UCSF, UCSD, Baylor, APA. Part 2; Bioline, Bristol-Myers Squibb, Elan, Lilly, Janssen Pharmaceutica, Merck, Novartis, Otsuka, Pfizer, Roche, Sepracor Dainippon-Sumitomo; U of So.Calif, UCSF, UCSD, Baylor. Part 3; Bioline, Bristol-Myers Squibb, Elan, Lilly, Janssen Pharmaceutica, Merck, Novartis, Otsuka, Pfizer, Roche,

Sepracor Dainippon-Sumitomo, U of So.Calif, UCSF, UCSD, Baylor. Part 4; Bioline, Bristol-Myers Squibb, Elan, Lilly, Janssen Pharmaceutica, Merck, Novartis, Otsuka, Pfizer, Roche, Sepracor Dainippon-Sumitomo, U of So.Calif, UCSF, UCSD, Baylor, APA.

43. Cognitive Enhancement in Euthymic Bipolar Patients with Pramipexole: A Placebo-Controlled Adjunctive Trial

Anil Malhotra, Raphael Braga, Charles Nnadi, Yaniv Shaya, Walter Stearns, Katherine Burdick*

Zucker Hillside Hospital-NSLIJHS, Glen Oaks, NY

Background: Patients with bipolar disorder suffer from significant cognitive impairment, even during periods of relative affective symptom remission. Persistent deficits in attention, verbal memory and executive functions contribute directly to functional disability in bipolar patients. Despite the clear impact of cognitive dysfunction on patients' quality of life, there have been few studies designed to target these symptoms for treatment.

Methods: We have conducted the first randomized, placebo-controlled, cognitive enhancement study in euthymic bipolar patients. The sample included 50 bipolar I patients who were randomized to either placebo or pramipexole, a dopamine d₂/d₃ receptor agonist, for an 8-week adjunctive trial. Study drug was flexibly dosed with a maximum pramipexole dose of 1.5 mg/day, which was achieved by all subjects. Patients completed a comprehensive neurocognitive battery, including measures of attention, processing speed, verbal learning and memory, and executive functions at baseline and again at the end of the study. Symptoms were monitored and side effects documented at weekly intervals throughout the study. Analyses were carried out using repeated measures analysis of variance initially for cognitive domain scores and subsequently for individual neurocognitive tasks. Covariates included baseline cognitive performance, change scores on depression and mania rating scales, and concomitant antipsychotic use.

Results: Thirty-five bipolar I patients (mean age = 42.7 ± 11.6 ; 66% female; 60% Caucasian) met strict criteria for euthymia at baseline (mean Hamilton Depression rating = 3.7 ± 4.0 ; mean Clinician Administered Mania rating = 2.3 ± 2.9) and completed the 8-week trial. Nineteen patients received placebo and 16 received pramipexole; groups were well-matched on demographic and clinical features, including baseline treatment regimen. Cognitive domain score analyses revealed a significant time x treatment group effect on 2 domains: attention/processing speed ($F = 5.5$; $p = 0.027$) and working memory ($F = 4.9$; $p = 0.035$). Patients assigned to pramipexole significantly improved over the 8-week trial in these domains while subjects in the placebo group showed no significant change. The treatment effect in the working memory domain was influenced by the presence of concomitant antipsychotic medications, such that the beneficial effect was significant only in those subjects not treated with antipsychotic agents. No effect of antipsychotics was observed for the attention/processing speed analysis. Effect size changes attributed to pramipexole on individual tests were in the moderate range with the strongest effect noted on WAIS Digits Backward ($d = 0.64$). There were no significant changes in mood ratings from baseline to week 8. Side effects more frequent in the pramipexole group than in the placebo group included restlessness, dry mouth, and headache.

Discussion: Our study provides the first evidence of significant cognitive enhancement in euthymic patients with bipolar disorder in the context of a randomized, placebo-controlled trial. Data suggest a beneficial effect of the dopamine agonist, pramipexole, on attention/processing speed and working memory when added to a standard mood-stabilizing regimen. Future studies will be important to address methodological challenges specific to cognitive outcome studies in bipolar illness, such as concomitant medications.

Disclosure: A. Malhotra: Part 1; Eli Lilly, Janssen, Vanda Pharmaceuticals, Sunovion. Part 2; Merck, BMS, PGx Health. R. Braga:

None. C. Nnadi: None. **Y. Shaya:** None. **W. Stearns:** None. **K. Burdick:** Part 1; Merck.

44. Efficacy of Iloperidone in the Short-Term Treatment of Schizophrenia: Meta-Analysis of Individual Patient Data from 4 Phase III Placebo- and Active-Comparator Controlled Trials

Leslie Citrome*, Stephen Stahl, Xiangyi Meng, Marla Hochfeld

Nathan S. Kline Institute for Psychiatric Research, Orangeburg, NY, University of California, San Diego, Carlsbad, CA

Background: Iloperidone (ILO) is a mixed D₂/5-HT₂ antagonist atypical antipsychotic approved for the treatment of schizophrenia. This meta-analysis assessed key efficacy outcomes *post hoc* from 4 phase III studies that evaluated the efficacy and safety of ILO vs placebo (PBO) with active controls in patients with schizophrenia after short-term treatment (4-6 weeks).

Methods: Subject-level data were pooled from 4 prospective, randomized, double-blind, PBO-controlled, multicenter trials of ILO in patients with schizophrenia aged 18-65 years. Active controls were included to confirm trial validity. The following treatments were evaluated (all dosed twice daily): ILO 4-8 mg/day; ILO 10-16 mg/day; ILO 20-24 mg/day; risperidone (RIS) 4-8 mg/day; haloperidol (HAL) 15 mg/day; ziprasidone (ZIP) 160 mg/day; or PBO. Outcomes of interest were change from baseline to endpoint in Brief Psychiatric Rating Scale (derived) (BPRSd), Positive and Negative Syndrome Scale (PANSS) Total (PANSS-T) score, and Positive (PANSS-P) and Negative (PANSS-N) subscales. Improvement is denoted by a positive number. An analysis of covariance (with treatment and study as factors, baseline as a covariate) was performed to compare changes between the treatment groups, based on a last-observation-carried-forward approach for the intent-to-treat (ITT) populations (i.e., all randomized patients who received at least 1 dose of study medication and had at least 1 efficacy measurement during treatment). The analysis included both direct and indirect comparisons, including pair-wise comparisons for ILO vs PBO, ILO vs RIS, ILO vs HAL, and ILO vs ZIP. Because of the different durations of the individual studies, PBO, HAL, and RIS data were examined out to 6 weeks; ZIP data were derived from a 4-week study.

Results: The ITT population (N = 2401) included n = 370, n = 494, and n = 424 for ILO 4-8, ILO 10-16, and ILO 20-24 mg/day, respectively; n = 294 for RIS; n = 114 for HAL; n = 144 for ZIP; and n = 561 for PBO. Main results for patients with schizophrenia only (n = 1941) are summarized below; complete results will be presented in detail, including the pair-wise comparisons, and outcomes for patients with either schizophrenia or schizoaffective disorder. BPRSd scores: Least squared mean (LSM) changes (with standard error) after 6 weeks' treatment were: 5.6 (0.81), 7.1 (0.65), and 8.1 (1.31) for ILO 4-8, ILO 10-16, and ILO 20-24 mg/day, respectively; 11.1 (0.88) for RIS; 8.9 (1.60) for HAL; and 3.8 (0.71) for PBO. LSM change at 4 weeks was 6.6 (1.16) for ZIP. PANSS-T scores: LSM changes from baseline to Week 6 were: 8.2 (1.36), 10.9 (1.09), and 13.0 (2.19) for ILO 4-8, ILO 10-16, and ILO 20-24 mg/day, respectively; 18.0 (1.47) for RIS; 14.4 (2.69) for HAL; and 5.6 (1.19) for PBO. LSM change at Week 4 was 11.1 (1.92) for ZIP. PANSS-P scores: LSM changes at Week 6 were: 3.3 (0.43), 4.1 (0.35), and 4.9 (0.70) for ILO 4-8, ILO 10-16, and ILO 20-24 mg/day, respectively; 6.9 (0.47) for RIS; 5.2 (0.86) for HAL; and 2.3 (0.38) for PBO. LSM change at Week 4 was 4.2 (0.61) for ZIP. PANSS-N scores: LSM changes at Week 6 were: 1.5 (0.35), 2.2 (0.28), 2.7 (0.57) for ILO 4-8, ILO 10-16, and ILO 20-24 mg/day, respectively; 3.5 (0.38) for RIS; 2.4 (0.70) for HAL; and 1.3 (0.31) for PBO. LSM change at Week 4 was 2.4 (0.51) for ZIP. **Safety:** Adverse events (AEs) leading to treatment withdrawal occurred in 5.1% of ILO patients and in 5.5% PBO, 7.6% HAL, 6.2% RIS, and 10.7% ZIP patients. Drug-related serious AEs occurred in 0.7% of ILO patients and in 1.4% PBO, 2.5% HAL, 2.0% RIS, and 0% ZIP patients.

Discussion: Consistent with product labeling, ILO 10-16 mg/day or ILO 20-24 mg/day demonstrated significant benefits over PBO in improving BPRSd and PANSS-T and subscale scores over 6 weeks.

Improvements in efficacy measures were generally greater with RIS than ILO, though ILO may offer advantages in terms of tolerability. ILO was efficacious and well tolerated in the short-term treatment of schizophrenia, particularly at doses approaching 24 mg/day (maximum recommended dose).

Disclosure: **L. Citrome:** Part 1; Astra Zeneca, Eli Lilly, Novartis, Pfizer, Sepracor, Merck. Part 2; Astra Zeneca, Eli Lilly, Merck, Novartis, Pfizer, Sepracor. Part 4; Astra Zeneca, Eli Lilly, Pfizer, Sepracor. **S. Stahl:** Part 1; Adams Coogler, Alkermes, Allergan, Arbor Scientia, Arena, Astra Zeneca, Azur, Biomarin, Bionevia, Biovail, Boehringer Ingelheim, BMS, Cambridge University Press, CME Solutions, Covance, Cypress Bioscience, Dianippon Sumitomo, Eisai, Eli Lilly, Endo Finnegan Henderson, Forest, Genomind, GSK, Great Point Partners, Health Wellness Partners, HealthLogix, Pierre Fabre, Innovision, Interactive Forums, Ionevia Pharma, Janssen, Jazz Pharmaceuticals, LaboPharm, Lundbeck, Marinus, Meiji, Merck, Neuronetics, Neuroscience Education Institute, Novartis, Noven, Nuvis, Ogilvy, Otsuka, PamLabs, Paragon, Pfizer, PGX Health, Propagate Pharma, Rexahn, Sanofi, Schering Plough, SciMed, Sepracor, Servier, Shire, SK Corporation, Solvay, Somaxon, State of California, Vanda, Vivus, Wyeth. Part 2; Arbor Scientia, Astra Zeneca, Biomarin, Boehringer Ingelheim, Cambridge University Press, Dianippon Sumitomo, Eisai, Eli Lilly, Endo, Forest Laboratories, Genomind, Innovision, Janssen, Labopharm, Lundbeck, Meiji, Neuroscience Education Institute, Novartis, PamLabs, Pfizer, Pierre Fabre, Sanofi, Schering Plough, Sepracor, Servier, Shire, SK Corporation, Somaxon, Vanda, Wyeth. Part 3; Arbor Scientia, Cambridge University Press, Neuroscience Education Institute, PamLabs. Part 4; Alkermes, Astra Zeneca, Boehringer Ingelheim, BMS, Cephalon, Dainippon Sumitomo, Eli Lilly, Forest, Janssen, Merck, Novartis, Organon, PamLabs, Pfizer, PGX Health, PriMed, Sanofi, Schering Plough, Sepracor, Servier, Shire, Takeda, Somaxon, Torrent, Vanda, Wyeth. **X. Meng:** Part 5; Novartis. **M. Hochfeld:** Novartis.

45. Substance Use Disorder Does Not Impair Recovery from Major Depression with Combination Antidepressant Treatment Compared to SSRI Monotherapy

Lori Davis*, Robert Howland, Sidney Zisook, Stephen Wisniewski, GK Balasubramani, Maurizio Fava, Madhukar Trivedi, A. John Rush

Veterans Affairs Medical Center, Tuscaloosa, AL

Background: Approximately one-third of outpatients seeking treatment for major depressive disorder (MDD) endorse concurrent substance use disorders (SUD). Outcome studies in the dual-diagnosed depressed patients have been mixed and few definitive studies are available to guide the clinician in treating depressed patients who are concurrently using alcohol or drugs. In the STAR*D study we found that, despite the ominous baseline clinical differences, there were no differences in most, but not all, of the first-line citalopram treatment outcomes between the MDD and the MDD + SUD groups. Compared to those without SUD, those with MDD and both alcohol and drug use were 42% less likely to achieve remission and experienced a significantly longer time to remission. Clinicians often turn to combinations of antidepressants when treatment resistance or relapse occurs.

Methods: To understand treatment outcomes for individuals with MDD and concurrent SUD, we examined the impact of concurrent SUD in the recovery of individuals with chronic and/or recurrent MDD following treatment with either a single agent SSRI versus two different combinations of two broader spectrum antidepressants from the multi-site study entitled "Combining Medications to Enhance Depression Outcomes" (CO-MED) COMED study. This multisite, single-blind, randomized COMED study compared the treatment effectiveness of s-citalopram (S-CIT) plus placebo with that of S-CIT plus bupropion-SR, and with that of mirtazapine plus venlafaxine-XR in a acute 12-week treatment followed by a 4-month continuation phase compared the longer-term outcomes in all participants who had

adequate benefit from the acute phase treatment. Participants were grouped by the presence/absence of SUD based on the participants' response on the Psychiatric Diagnostic Screening Questionnaire.

Results: Of the 664 evaluable participants, 13.1% (n = 87) were classified as having concurrent SUD symptoms (2.3% having both drug and alcohol, 3% having drug only, and 7.8% having alcohol only). Compared to SUD- participants, those with SUD+ were significantly more likely to be male and to have current suicidal thoughts/plans, a history of suicide attempts, a greater number of suicide attempts, a greater lifetime severity of suicidality, and a higher number of concurrent psychiatric Axis I disorders, namely an increased number of concurrent anxiety disorders. Compared to SUD- participants, those with SUD+ were of lower body mass index and had lower rates of diabetes. The treatment outcomes were similar between the between the SUD- and SUD+ groups in terms dose, time in treatment, response or remission for the 12-week and 28-week study intervals. The 12-week outcome measures by substance abuse and treatment did not significantly differ. For both groups, 17.4% to 38.5% achieved remission and 43.5% to 52.9% achieved response, based on self-report scale.

Discussion: The main finding of this study is that, despite baseline differences in lifetime suicidality and increased number of concurrent Axis I disorders, the presence of concurrent SUD in persons with chronic or recurrent MDD does not appear to negatively impact recovery when treated with either single agent or combination antidepressants. Secondly, the recovery, as defined by response or remission, in individuals suffering from chronic or recurrent MDD with or without SUD does not seem to differ between SSRI versus combination antidepressant treatment in short or long term treatment. Finally, the most important hazard imposed by SUD in this sample is that of increased lifetime suicidality, possibly providing hope that treatment for the depression and SUD may reduce the risk of fatal suicide. Our findings of equivalent response and remission rates to single agent or combination antidepressant medication treatment in individuals with chronic and/or recurrent MDD with or without SUD supports an emerging and important paradigm shift in clinical care. The striking burden of suicidality imposed by the comorbidity of SUD is clear in this and other studies and should heighten the urgency to assertively treat depression in patients who are also using drugs and alcohol. These findings may be useful for guiding the treatment of depression in persons with chronic and/or recurrent MDD and concurrent SUD in primary care and psychiatric clinics. However, since the subjects were recruited from a psychiatric or primary care clinic, the results may not be generalizable to a substance abuse treatment clinic.

Disclosure: L. Davis: Part 1; Eli Lilly, AstraZeneca. Part 4; AstraZeneca, Bristol-Myers Squibb. R. Howland: Medtronic, Cyberonics, AstraZeneca. S. Zisook: PamLab. S. Wisniewski: Part 1; Cyberonics Inc., Bristol-Myers Squibb, Singapore Clinical Research Institute, Dey Pharmaceuticals, Venebio, Dey. G. Balasubramani: None. M. Fava: Abbott Laboratories, Alkermes, Aspect Medical Systems, Astra-Zeneca, BioResearch, BrainCells, Inc, Bristol-Myers Squibb Company, Cephalon, Clinical Trial Solutions, LLC, Eli Lilly & Company, Forest Pharmaceuticals, Inc, Ganeden, GlaxoSmithKline, J&J Pharmaceuticals, Lichtwer Pharma GmbH, Lorex Pharmaceuticals, NARSAD, NCCAM, NIDA, NIMH, Novartis, Organon Inc, PamLab, LLC, Pfizer, Inc, Pharmavite, Roche, Sanofi-Aventis, Shire, Solvay Pharmaceuticals, Inc, Synthelabo, Wyeth-Ayerst Laboratories, Affectis Pharmaceuticals AG, Amarin, Aspect Medical Systems, Auspex Pharmaceuticals, Inc, Bayer AG, Best Practice Project Management, Inc, BioMarin Pharmaceuticals, Inc, Biovail Pharmaceuticals, Inc, Cephalon, CNS Response, Compellis, Cypress Pharmaceuticals, Dov Pharmaceuticals, Eisai, Inc, EPIX Pharmaceuticals, Euthymics Bioscience, Inc, Fabre-Kramer Pharmaceuticals, Inc, Grunenthal GmbH, Janssen Pharmaceutica, Jazz Pharmaceuticals, Knoll Pharmaceutical Company, Labopharm, Lorex Pharmaceuticals, Lundbeck, MedAvante, Inc, Merck, Methylation Sciences, Neuronetics, Nutrition 21, PharmaStar, Precision Human Biolaboratory, PsychoGenics, Psylin Neuroscience, Inc, Ridge

Diagnostics, Inc, Sepracor, Schering-Plough, Somaxon, Somerset Pharmaceuticals, Takeda, Tetragenex, TransForm Pharmaceuticals, Inc, Transcept Pharmaceuticals, Vanda Pharmaceuticals, Inc, EnVivo Pharmaceuticals, Clintara, LLC, Covidien, RCT Logic, LLC, CeNeRx BioPharma, GenOmni, LLC, Prexa Pharmaceuticals, Rexahn Pharmaceuticals, Sepracor, Inc. Part 4; Eli Lilly, GlaxoSmithKline, Bristol-Myers Squibb Company, PamLab, LLC, Sanofi-Aventis, Pfizer, Inc, AstraZeneca, Johnson & Johnson Pharmaceutical Research & Development, Novartis, Healthcare Technologies, BrainCells, Inc. M. Trivedi: Part 1; Abbott, Abdi Brahim, Akzo (Organon), AstraZeneca, Bristol-Myers Squibb, Cephalon, Eli Lilly, GlaxoSmithKline, Forest, Fabre-Kramer, Janssen, Meade Johnson, Johnson & Johnson, Neurogenetics, Parke-Davis, Pfizer, Sepracor, VantagePoint, Wyeth. Part 4; Corcept, Cyberonics, Novartis, Merck, Pharmacia & Upjohn, Predix, Solvay, and Targacept. A. Rush: Part 1; Advanced Neuromodulation Systems, AstraZeneca, Best Practice Project Management, Bristol-Myers Squibb, Cyberonics, Forest, Gerson Lehman Group, GlaxoSmithKline, Jazz, Magellan Health Services, Merck, Neuronetics, Novartis, Ono, Organon, Otsuka, Pam Labs, Pfizer, Trancept, The Urban Institute, and Wyeth.

46. The Relationship between Interview Quality and Scoring Accuracy: Evaluation of the Rater Applied Performance Scale Danielle Popp, Michael Detke*, Janet Williams

MedAvante Research Institute, Hamilton, NJ

Background: Quality of interviews may be an important contributor to the high number of failed CNS clinical trials. Recent advances in the measurement of interviewer skill allow for an examination of the effects of interview quality on signal detection¹. Kobak and colleagues demonstrated that patients with higher quality interviews are more likely to distinguish an effective drug from placebo, suggesting that CNS clinical trials could benefit from ongoing monitoring of interview quality². The current study examines the relationships between interview quality and score accuracy between site raters and a team of calibrated, blinded independent clinician reviewers. The study also seeks to identify which areas of interview quality are most closely related to scoring accuracy, which has important implications for rater training and remediation.

Methods: Data were pooled from two ongoing clinical trials of Major Depressive Disorder (MDD). Site raters performed MADRS or HAM-D assessments on subjects. All site rater assessments were audio taped and uploaded to a central server. A group of calibrated, blinded independent clinician reviewers scored a subset of downloaded site rater assessments selected according to an *a priori* algorithm. The reviewers also rated interviews on a 1 to 4 scale on 5 domains of the Rater Applied Performance Scale (RAPS): Adherence, Follow Up, Clarification, Neutrality, and Rapport. Independent reviewers' scoring of the scale items and the RAPS were completed and locked prior to revealing site rater scores.

Results: 1017 interviews across 2 MDD studies were analyzed to examine relationships between RAPS ratings and scoring accuracy. As the two studies relied on different scales, all total scores were standardized to z-scores ($M=0$, $SD=1$). Scoring accuracy was computed as the difference between standardized site rater scores and standardized reviewer scores. Cronbach's alpha demonstrated good internal consistency across the 5 domains of the RAPS (i.e., Adherence, Follow Up, Clarification, Neutrality, and Rapport; $\alpha=.75$). Of the 1017 interviews, 49.4% were categorized as Good or Excellent quality (average of 5 RAPS domains ≥ 3), 49.2% were Fair (average of 5 RAPS domains 2.0 to < 3.0), and 1.5% were Unsatisfactory (average of 5 RAPS domains < 2). Intraclass correlations (ICCs) between site rater and independent reviewer scores were computed for interviews across different interview quality categories. Higher quality interviews had higher ICCs (Unsatisfactory = .39, Fair = .88, Good/Excellent = .96). For baseline and screening visits during which cut-off scores were applied (n = 599), scoring accuracy was significantly

correlated with all 5 individual RAPS domains and overall RAPS score (computed as the rater's average score across all 5 items) (all r 's $>-.097$, all p 's $<.018$). In order to examine the predictive relationships of each individual RAPS domain, a multiple regression was performed demonstrating that 3 domains of the RAPS significantly predicted scoring accuracy: Adherence ($\beta = -.173$, $p <.001$), Follow Up ($\beta = -.162$, $p <.001$), and Clarification ($\beta = -.115$, $p <.05$). Higher scores on each of these domains predicted better scoring accuracy (i.e., smaller differences between site and reviewer scores). At follow up visits ($n = 417$), scoring accuracy was significantly correlated with the overall RAPS average and 4 of the RAPS domains: Follow Up, Clarification, Neutrality, and Rapport (all r 's $>-.098$, all p 's $<.046$). However, a multiple regression revealed none of the RAPS domains significantly predicted scoring accuracy. Additional data are currently being collected.

Discussion: Interview quality is strongly related to scoring accuracy across MDD studies. The Rater Applied Performance scale is not only a reliable measure of interview quality but also predicts scoring accuracy. Unsatisfactory quality interviews have lower scoring accuracy which may contribute to decreased signal detection and the high rate of CNS clinical trial failures. The RAPS is a useful tool in determining which interviews are of poor quality and identifying which domains are more important for scoring accuracy. The current study demonstrates the importance of assessing interview quality throughout a CNS trial. References: ¹Lipsitz, J., Kobak, K. A., Feiger, A., Sikich, D., Moroz, G., & Engelhardt, A. (2003). The Rater Applied Performance Scale (RAPS): Development and reliability. *Psychiatry Research*, *124*, 147-155. ²Kobak, K. A., Feiger, F., & Lipsitz, J. (2007). Impact of interview quality on signal detection in clinical trials. *American Journal of Psychiatry*, *162*, 628.

Disclosure: D. Popp: Part 1; MedAvante. Part 2; MedAvante. Part 3; MedAvante. Part 5; MedAvante. M. Detke: Part 1; MedAvante, Eli Lilly. Part 2; MedAvante, Eli Lilly. Part 3; MedAvante, Eli Lilly. Part 5; MedAvante, Eli Lilly. J. Williams: Part 1; MedAvante. Part 2; MedAvante. Part 3; MedAvante. Part 5; MedAvante.

47. Effectiveness of Adjunctive Aripiprazole Is Not Dependent on Antidepressant Therapy History: Pooled Data from Three Clinical Trials (CN138-139, CN138-163, CN138-165)

David L Dunner*, Kimberly Laubmeier, George Manos, Robert Forbes, Ross Baker

Center for Anxiety and Depression, Mercer Island, WA

Background: Current guidelines recommend switching antidepressant therapy (ADT) for patients showing minimal or no response to an optimized ADT, and augmenting for patients with partial response¹. However, it is unclear whether there is a differential effect of switching between SSRIs (within class) or from an SSRI to a non-SSRI (between class) as second step therapy prior to adjunctive use of an atypical antipsychotic. Secondary analysis of an augmentation study suggested that patients who have failed to respond adequately to an initial SSRI and a between class switch to an SNRI may respond differentially to augmentation with an atypical antipsychotic. The aripiprazole MDD clinical trial program, consisting of three nearly identical trials, provides a unique dataset for evaluating the effect of ADT history and comparing outcomes in patients switched between or within classes of ADT^{2,3}.

Methods: Patients with a history of 1 to 3 failed trials of ADT were switched to another ADT monotherapy and enrolled into a prospective trial for 8 weeks. The choice of ADT switch was up to investigator discretion. Inadequate responders to the ADT monotherapy (<50% reduction HAM-D₁₇ total, HAM-D₁₇ ≥ 14 and Clinical Global Impressions -Improvement [CGI-I] ≥ 3 at the end of the 8 weeks) were then randomized to treatment with either aripiprazole (2-20 mg/day) or placebo adjunctive to their ADT. The primary efficacy endpoint was the mean change in Montgomery-Asberg Depression Rating Scale (MADRS) total score from end of prospective treatment to

endpoint-week 6 (LOCF). Remission was defined as MADRS total score ≤ 10 at endpoint (LOCF).

Results: Four hundred sixteen patients were switched within class, and 623 between classes. Notably among between class switches, 149 patients switched from bupropion to an SSRI and 78 to an SNRI. The mean change from end of Phase B (Prospective treatment phase) in MADRS total score was significant both for switch within class (aripiprazole, $n = 206$, -9.68 vs. placebo, $n = 210$, -6.68 ; difference = -3.0 , 95%CI:-4.60,-1.40) and switch between class (aripiprazole, $n = 319$, -9.08 vs. placebo, $n = 304$, -6.17 ; difference = -2.91 ; 95% CI:-4.20, -1.61). Most of the treatment effect occurred early, within the first 2 weeks. The remission rate for switch within class was 29.1% aripiprazole vs. 18.1% placebo compared with switch between class 29.2% aripiprazole vs. 15.8% placebo.

Discussion: Data from the aripiprazole clinical trial program suggest adjunctive aripiprazole is equally effective for patients who have been previously switched between ADT classes, or within an ADT class. Thus, inadequate response to ADT monotherapy, and not treatment history, can provide guidance to clinicians considering augmentation of an ADT with aripiprazole. These data also suggest that adjunctive aripiprazole treatment may be considered after the initial ADT failure and, as evidenced by the rapidity of response (within 2 weeks), it may be a quicker strategy to attain remission than switching ADTs or other forms of adjunctive therapy. References: 1. Nutt DJ, Davidson JR, Gelenberg AJ, et al. International consensus statement on major depressive disorder. *J Clin Psychiatry*. 2010;71[suppl E1]:eo8. 2. Thase ME, Trivedi MH, Nelson JC, et al. Examining the efficacy of adjunctive aripiprazole in major depressive disorder: a pooled analysis of 2 studies. *Prim Care Companion J Clin Psychiatry* 2008;10(6):440-447. 3. Berman RM, Fava M, Thase ME, et al. Aripiprazole augmentation in major depressive disorder: a double-blind, placebo-controlled study in patients with inadequate response to antidepressants. *CNS Spectr*. 2009;14:197-206.

Disclosure: D. Dunner: Part 1; Eli Lilly, Wyeth, Cyberonics, Pfizer, Jazz Pharmaceuticals, Sanofi-Aventis, Janssen, Bristol-Myers Squibb, Neuronetics, AstraZeneca. Part 2; Wyeth, Cyberonics, Jazz Pharmaceuticals, AstraZeneca. Part 4; Neuronetics, Cyberonics. K. Laubmeier: Part 1; Bristol-Myers Squibb. Part 5; Bristol-Myers Squibb. G. Manos: Part 1; Bristol-Myers Squibb. Part 5; Bristol-Myers Squibb. R. Forbes: Part 1; Otsuka Pharmaceutical & Commercialization, Inc. Part 5; Otsuka Pharmaceutical & Commercialization, Inc. R. Baker: Part 1; Bristol-Myers Squibb. Part 5; Bristol-Myers Squibb.

48. L-Methylfolate Augmentation of Selective Serotonin Reuptake Inhibitors (SSRIs) for SSRI-Resistant Major Depressive Disorder: Results of Two Randomized, Double-Blind Trials

Maurizio Fava*, Richard Shelton, John Zajecka, Karl Rickels, Alisabet Clain, Lee Baer, David Schoenfeld, Erik Nelson, James Barbee, Bruce Lydiard, David Mischoulon, Jonathan Alpert, Sidney Zisook, George Papakostas

Massachusetts General Hospital Boston, MA

Background: The present studies are two identical multi-center, randomized, double-blind, placebo-controlled trials of augmentation of SSRIs with the folate metabolite L-methylfolate (Deplin), conducted in a sequenced fashion to allow for the determination of the optimal dosing of L-methylfolate in this population. To enhance signal detection, a novel study design approach (sequential parallel comparison design or SPCD) was employed in both studies.

Methods: In Study One (TRD-1 Study), 148 outpatients [103 Women (70%), Mean Age $47.9 +/- 11.6$, Mean Baseline HRDS-17 $19.7 +/- 4.7$] with SSRI-resistant major depressive disorder (MDD) were enrolled in a multi-center, 60-day, double-blind SPCD study, which was divided into two, 30-day periods (phases 1 and 2). Patients were randomized, in a 2:3:3 fashion, to receive either L-methylfolate for 60 days (7.5 mg/d in phase 1, 15 mg/d in phase 2), placebo for 30 days in phase 1 followed by L-methylfolate for 30 days (7.5 mg/d) in phase 2, or placebo for 60 days

(in both phases). In Study Two (TRD-2 Study), 75 outpatients [53 Women (71%), Mean Age 48.49 +/-12.169, Mean Baseline HRSD-17 21.28 +/-3.94] with SSRI-resistant MDD were enrolled in a multicenter, 60-day, double-blind SPCD study, which was divided into two, 30-day periods (phases 1 and 2). Patients were randomized, in a 2:3:3 fashion, to receive either L-methylfolate for 60 days (15 mg/d in both phases), placebo for 30 days in phase 1 followed by L-methylfolate for 30 days (15 mg/d) in phase 2, or placebo for 60 days (in both phases). SSRI doses were kept constant during the double-blind phases of both studies. The main outcome measures for both studies were the change in total scores of the 17-item Hamilton Depression Rating Scale (HDRS-17) and the response rates (50% or greater reduction in HDRS-17), according to the method developed for SPCD studies, pooling the data from all study participants in phase 1 and the data from placebo non-responders only in phase 2.

Results: In the TRD-1 Study, L-methylfolate 7.5 mg/d was not found to be more effective than placebo as an SSRI augmentor according to the SPCD analyses (weighed difference in response rates, approximately, 3% in favor of placebo). While the response rate to placebo in phase 2 was only 9.0%, the response rate for L-methylfolate (7.5 mg/d) non-responders during phase 1 who underwent an increase in L-methylfolate dose (15 mg/d) during phase 2 was 24.0% ($p = 0.1$). In the phase 1 of the TRD-2 Study, 37% (7/19) of the patients on L-methylfolate (15 mg/d) responded and 18% (11/56) of the placebo patients responded, while in phase 2 among placebo non-responders, the response rates were 28% (5/18) on L-methylfolate (15 mg/d) and 9.5% (2/21) on placebo. When these phase 1 and 2 data are analyzed using the method described for the SPCD, the pooled difference between active treatment and placebo is statistically significant ($p = 0.0399$). In terms of tolerability, among all subjects in phase 1 and placebo non-responders in phase 2, the rates of spontaneously reported AEs appear to be rather comparable between L-methylfolate and placebo in both studies. In the same population, the rates of study discontinuation were also rather comparable (11.3% in TRD-1 and 10.8% in TRD-2 for L-methylfolate and 12.0% in TRD-1 and 10.4% in TRD-2 for placebo).

Discussion: These two studies suggest that 15 mg/d of L-methylfolate may be a safe and effective augmentation strategy for patients with inadequate response to SSRI treatment.

Disclosure: M. Fava: Part 1; Abbott, Alkermes, Astra-Zeneca, BioResearch, BrainCells, BMS, Clinical Trial Solutions, Eli Lilly, Forest, Ganeden, GSK, J & J Pharmaceuticals, Lichtwer Pharma GmbH, NARSAD, NCCAM, NIDA, NIMH, Novartis, Organon, PamLab, Pfizer, Pharmavite, Roche, Sanofi-Aventis, Shire, Solvay, Synthelabo, Wyeth-Ayerst, Affectis, Amarin, Aspect Medical Systems, Auspex, Bayer AG, Best Practice Project Management, Inc, BioMarin, Biovail, Cephalon, CNS Response, Compellis, Cypress, Dov, Eisai, EPIX, Euthymics Bioscience, Fabre-Kramer, Grunenthal GmbH, Janssen, Jazz, Knoll, Labopharm, Lorex, Lundbeck, MedAvante, Merck, Methylation Sciences, Neuronetics, Nutrition 21, PharmaStar, Precision Human Biolaboratory, PsychoGenics, Psylin Neuroscience, Ridge Diagnostics, Sepracor, Schering-Plough, Somaxon, Somerset, Takeda, Tetragenex, TransForm, Transcept, Vanda, EnVivo, Clintara, Covidien, RCT Logic, CeNeRx BioPharma, GenOmind, Prexa, Rexahn, Sepracor. Part 4; Lilly, GSK, BMS, PamLab, Sanofi-Aventis, Pfizer, AstraZeneca, Johnson & Johnson, Novartis, Healthcare Technologies, BrainCells. R. Shelton: Part 1; Eli Lilly, Evotec AG, Forest, Gideon Richter, Janssen, Medtronic, Novartis, Otsuka, PamLab, Pfizer, Repligen, Sierra Neuropharmaceuticals, St. Jude Medical. Part 4; Eli Lilly, Forest, Janssen, Novartis, Otsuka, PamLab, Pfizer, Repligen, St. Jude Medical. J. Zajecka: Part 1; Abbott, BMS/Otsuka, Lilly, Novartis, AstraZeneca, PamLab, Pfizer, Takeda, Boehringer-Ingelheim, CNS Response, McNeil, NIMH, GSK, Covidien, Cyberonics, Cheryl T. Herman Charitable Foundation. Part 2; AstraZeneca, BMS/Otsuka, Lilly, PamLab, NIMH. Part 3; AstraZeneca, BMS/Otsuka, Lilly. Part 4; Astra Zeneca, Boehringer-Ingelheim, BMS/Otsuka, CNS Response, Cyberonics, GSK, McNeil, NIMH, PamLab, Pfizer, Takeda. K. Rickels: Part 1; Pfizer. Part 4; BMS, Epix, Genaissance, NIMH, PamLab, Pfizer, Wyeth. A. Clain: None.

L. Baer: None. D. Schoenfeld: Part 1; Employer has a patent on the experimental design in this clinical trial. I get a share of royalties if any. E. Nelson: None. J. Barbee: Lilly, BMS, AstraZeneca, Merck, Novartis. Part 2; Lilly, BMS, AstraZeneca. B. Lydiard: None. D. Mischoulon: Part 1; Laxdale (Amarin), Nordic Naturals, Ganeden, SwissMedica, BMS, PamLab, Virbac, Reed Medical Education, Royalties from Back Bay Scientific, Royalties from Lippincott Williams & Wilkins. Part 4; Bowman Family Foundation. J. Alpert: PamLab, Bristol-Myers Squibb, GlaxoSmithKline, Pfizer, Healthcare Technologies, AstraZeneca, BrainCells, Eli Lilly, Sanofi-Aventis. S. Zisook: PamLab. G. Papakostas: Part 1; Abbott, AstraZeneca, BMS, Lilly, GSK, Lundbeck, Otsuka, PAMLAB, Pfizer, Pierre Fabre, Shire, & Wyeth, Forest, NIMH, & Ridge Diagnostics. Part 2; GSK, BMS, AstraZeneca, Lundbeck Janssen-Cilag, Otsuka. Part 3; GSK, BMS, AstraZeneca, Lundbeck Janssen-Cilag, Otsuka. Part 4; Research support paid to hospital (not me) from BMS, Forest, NIMH, PAMLAB, Pfizer, & Ridge Diagnostics.

49. A Multicenter Effectiveness Study of Paliperidone Palmitate vs Oral Antipsychotics for People With Schizophrenia Recently Released From Jail: Study Rationale and Methodology

Reuven Ferziger*, Larry Alphs, Norris Turner, Lian Mao, Stephen Rodriguez, Lisa Dixon, John Davis, Joseph Hulihan

Ortho-McNeil Janssen Scientific Affairs, LLC, Titusville, NJ

Background: The overrepresentation of people with serious mental illness (SMI) in US jails and prisons is an important public mental health problem [1-3]. According to a recent analysis of US Department of Justice data there are three times as many people with SMI in correctional settings than in psychiatric inpatient facilities [4]. After release from incarceration, community reentry is challenged by barriers to obtaining essential healthcare, social services, and financial resources, perpetuating a revolving door of incarceration, followed by failed community reentry and recidivism to jail or prison [5]. Although untreated psychotic illness may be a significant variable, no studies comparing the effectiveness of psychopharmacologic treatments in individuals with schizophrenia following release from jail have been conducted [6]. A recently initiated clinical study compares a monthly, long-acting injectable antipsychotic with daily oral antipsychotics in delaying time to reentry failure in patients with schizophrenia who have been recently released from jail. The study is being conducted at approximately 50 US sites. There is extensive site variation due to regional differences in the mental health systems and available services for this population among the states and counties where the sites are located. This both reflects the real world nature of the study and poses methodological challenges for the study design and analysis. We report on the nature of the problem, the study rationale, and methodological questions encountered in designing a psychopharmacology intervention trial that addresses public health and clinical dimensions of a problem frequently encountered by people with schizophrenia.

Methods: This is a prospective, randomized, open-label, multicenter, effectiveness study comparing paliperidone palmitate with oral antipsychotics in subjects with schizophrenia who were recently released from jail and who were incarcerated at least twice within the preceding 24 months. The study consists of a 14-day screening phase and 15-month treatment phase. Before randomization, the investigator/clinicians and the subjects identify individually suitable oral antipsychotics from a list of seven commonly prescribed oral antipsychotics (aripiprazole, haloperidol, olanzapine, paliperidone, perphenazine, quetiapine, or risperidone). At randomization (1:1), subjects are assigned treatment to paliperidone palmitate or to one of the oral antipsychotic agents that were prespecified as appropriate therapy for that individual. The primary endpoint is time to occurrence of a treatment failure event, defined as arrest; hospitalization; suicide; discontinuation of antipsychotic treatment due to inadequate efficacy, safety, or tolerability; treatment supplementation with another antipsychotic due to inadequate efficacy; or an increase in the level of psychiatric services in order to prevent imminent psychiatric

hospitalization. Other measures include the Clinical Global Impressions-Severity scale, Medication Satisfaction Questionnaire, Activities of Daily Living, Sheehan Disability scale, alcohol and drug abuse section of the Addiction Severity Index, Personal and Social Performance scale, and resource utilization measurements.

Discussion: This is a comparative effectiveness study to determine whether time to treatment failure for people with schizophrenia recently released from jail differs between treatment with paliperidone palmitate, a long-acting atypical injectable antipsychotic, and commonly prescribed oral antipsychotics. Endpoints were chosen to address meaningful clinical and public health outcomes. Supported by Ortho-McNeil Janssen Scientific Affairs, LLC. References: 1. Council of State Governments. Criminal Justice/Mental Health Consensus Project Report. June 2002. http://consensusproject.org/the_report 2. Committee on Psychiatry and the Community. Jailing is failing people with mental illness. *Psychiatr Serv.* 2009;60(6):723 3. Steadman HJ, et al. Prevalence of serious mental illness among jail inmates. *Psychiatr Serv.* 2009;60(6):761-5 4. Torrey EF, et al. More Mentally Ill Persons Are in Jails and Prisons Than Hospitals: A Survey of the States. Treatment Advocacy Center and the National Sheriff's Association, May 2010 http://www.treatmentadvocacycenter.org/storage/tac/documents/final_jails_v_hospitals_study.pdf 5. Baillargeon J, et al. Psychiatric disorders and repeat incarcerations: the revolving prison door. *Am J Psychiatry.* 2009;166(1):103-9 6. Medline and clinicaltrials.gov searches, August 22, 2010.

Disclosure: R. Ferziger: Part 5; Ortho-McNeil Janssen Scientific Affairs, LLC. L. Alphs: Employee, Ortho-McNeil Janssen Scientific Affairs, LLC, Johnson & Johnson stockholder. N. Turner: Part 1; Ortho-McNeil Janssen Scientific Affairs, LLC. Part 2; Ortho-McNeil Janssen Scientific Affairs, LLC. Part 5; Ortho-McNeil Janssen Scientific Affairs, LLC. L. Mao: Employee, stockholder: Johnson & Johnson PRD, LLC. S. Rodriguez: Part 1; Johnson & Johnson. Part 5; Ortho-McNeil Janssen Scientific Affairs, LLC. L. Dixon: University of Maryland School of Medicine. J. Davis: None. J. Hulihan: Johnson & Johnson PRD, LLC.

50. Risk For Discontinuation Due To Adverse Events, Extrapyramidal Side Effects, Somnolence, and Weight Gain of Ziprasidone Monotherapy Versus Placebo During Acute Treatment Of Schizophrenia, Mania or Bipolar Depression

Joseph Calabrese, Keming Gao*, Elizabeth Pappadopoulos, Onur Karayal, Kolluri Sheela

Case Western Reserve University, Cleveland, OH, University Hospitals Case Medical Center, Cleveland, OH

Background: Most antipsychotics are approved for use in both schizophrenia and bipolar disorder. Comparisons of the safety data of antipsychotics in schizophrenia and bipolar disorder revealed that there were differential sensitivities and tolerabilities to different antipsychotics between patients with schizophrenia and bipolar disorder, and between patients with bipolar mania and bipolar depression. [1,2] For instance, patients with bipolar mania have a higher risk for EPS than those with schizophrenia when treated with haloperidol and atypical antipsychotics. [1] In quetiapine-XR studies, the risk for discontinuations due to adverse events (DAEs) and reported somnolence of quetiapine-XR related to placebo was higher in patients with bipolar depression than those with schizophrenia or mania. [2] It remains unclear if patients with schizophrenia or bipolar disorder have differential sensitivities and tolerabilities to ziprasidone. Here we compare the risk for DAEs, EPS, somnolence, and weight gain across Pfizer-sponsored studies of ziprasidone in schizophrenia and bipolar disorder.

Methods: Nine double-blind, placebo controlled, short-term [$/=7\%$ weight gain of ziprasidone relative to placebo during the treatment of these psychiatric conditions were calculated. The numbers needed to treat to harm (NTTH) of these variables were estimated with 95% confidence interval (CI) to reflect the magnitude of variance.

Results: There was no significant difference in the DAEs risk between ziprasidone and placebo in the treatment of schizophrenia with a rate of 4.1% vs. 2.1% and a NNTH of 52 (95% CI 25 to -115), bipolar mania with a rate of 5.5% vs. 3.1% and a NNTH of 43 (95% CI 19 to -78), and bipolar depression with a rate of 14.6% vs. 10.2% and a NNTH of 23 (95% CI 12 to -1488). For akathisia, there was no significant difference between ziprasidone and placebo in schizophrenia with a rate of 8.1% vs. 6.6% and a NNTH of 66 (95% CI 21 to -40). However, in bipolar mania, the risk for akathisia was significantly higher for ziprasidone relative to placebo with a rate of 15.3% vs. 4.5% and a NNTH of 9 (95% CI 7 to 16). Similarly, in bipolar depression, the risk for akathisia was also significantly higher for ziprasidone relative to placebo with a rate of 3.1% vs. 0.8% and a NTTH of 45 (95% CI 24 to 348). Risk for overall EPS of ziprasidone relative to placebo was significantly higher in schizophrenia and mania, but not in bipolar depression with a rate of 4.6% vs. 1.1% and a NNTH of 20 (95% CI 19 to 100) in schizophrenia, 15.1% vs. 4.9% and a NNTH of 10 (95% CI 7 to 18) in bipolar mania, and 1.2% vs. 0.5% and a NNTH of 116 (95% CI 50 to -104) in bipolar depression, respectively. Risk for reported somnolence of ziprasidone relative to placebo was significantly higher in 3 psychiatric conditions, with a rate of 14.4% vs. 6.6% and a NNTH of 13 (95% CI 9-29) in schizophrenia, 24.1% vs. 8.5% and a NNTH of 6 (95% CI 5 to 10) in mania, and 15.4% vs. 3.6% and a NNTH of 8 (95% CI 6 to 12) in bipolar depression. On the other hand, the risk for $>/=7\%$ weight gain of ziprasidone relative placebo was significantly higher in schizophrenia with a rate of 9.1% vs. 3.7% and a NNTH of 17 (95% CI 11 to 48), but not in mania with a rate of 6.5% vs. 3.1% and a NNTH of 29 (95% CI 15 to -96), or bipolar depression with a rate of 2.3% vs. 1.0% and NNTH of 76 (95% CI 30 to -124).

Discussion: At the studied doses, patients with schizophrenia, mania, or bipolar depression tolerated ziprasidone well, but these patients had different risk profiles. Patients with bipolar mania had the smallest NNTH for akathisia, overall EPS, and somnolence. However, patients with schizophrenia had the smallest NNTH for $>/=7\%$ weight gain. Patients with bipolar depression only had significant high risk for somnolence with ziprasidone relative to placebo, which is inconsistent with some previous findings that patients with bipolar depression had lowest tolerability and highest sensitivity to antipsychotics compared to those with schizophrenia or bipolar mania.[1,2] This inconsistency may arise from flexible dosing with a wide range of ziprasidone doses in bipolar depression studies. References: 1. Gao K et al. Antipsychotic-induced extrapyramidal side effects in bipolar disorder and schizophrenia: a systematic review. *J Clin Psychopharmacol.* 2008 Apr;28(2):203-9. 2. Wang Z et al. Comparisons of the Tolerability and Sensitivity of Quetiapine-XR in the Acute Treatment of Schizophrenia, Bipolar Mania, Bipolar Depression, Major Depressive Disorder, and Generalized Anxiety Disorder. *Int J Neuropsychopharmacol* (in press).

Disclosure: J. Calabrese: Part 1; AstraZeneca, Cephalon, Dainippon, Forest Pharmaceuticals, France Foundation, GlaxoSmithKline, J & J, Janssen, Lundbeck, Neurosearch, OrthoMcNeil, Otsuka, Merck, Pfizer, Repligen, Sanofi, Schering-Plough, Servier, Synosia, Takeda, Wyeth. Part 2; AstraZeneca, GlaxoSmithKline, Merck. Part 3; AstraZeneca, GlaxoSmithKline. Part 4; Takeda. K. Gao: Part 1; Pfizer Inc., Schering-Plough. Part 4; AstraZeneca, NARSAD. E. Pappadopoulos: Part 1; Pfizer Inc.. Part 2; Pfizer Inc.. Part 5; Pfizer Inc. O. Karayal: Part 1; Pfizer Inc.. Part 5; Pfizer Inc. K. Sheela: Part 1; Pfizer Inc.. Part 5; Pfizer Inc.

51. The First Time is Best: Highest Remission Rate to SSRI Antidepressant in First Episode of Treatment for Depression

Steven Garlow*, Philip Ninan, Charles Nemerooff

Emory University School of Medicine, Atlanta, GA

Background: The immediate goal of treatment of unipolar depressive illness is full remission, which is generally defined as a period during which the patient does not meet syndromal criteria for a depressive

episode and has only negligible residual symptoms. Practically, the definition of remission is based on a minimal score on one of the standard depression symptom severity scales. Understanding the circumstances that lead to remission and hence the potential for long-term recovery is critical towards improving overall treatment outcomes for depressive disorders.

Methods: This is a secondary analysis of data that were collected as part of a double blind, placebo-controlled 8-week trial exploring the efficacy of combination treatment of sertraline (50-200 mg/d) plus triiodothyronine (T₃, 25-50 µg/d) or placebo in adult outpatients with major depressive disorder. (Results have been presented elsewhere, but T₃ did not separate from placebo in any efficacy measure). Diagnostic inclusion criteria were a DSM-IV defined diagnosis of major depressive disorder (MDD), without psychotic features. Symptom severity inclusion criteria were of 21-item Hamilton Rating Scale of Depression (HRSD-21) of 18 or greater at screening and baseline assessment one week later. There was a one-week single blind, placebo lead-in period between screening and baseline assessments, and eligible patients started treatment at the baseline visit. Medication adjustment occurred at weeks 1, 2, 3, 4, and 6. The HRSD-21, Montgomery-Asberg Depression Rating Scale (MADRS) and Clinical Global Impression (CGI) scales were utilized to assess depressions severity at all visits. Remission was defined as HRSD-21<8 at final assessment. A last observation carried forward (LOCF) design was utilized and those patients who took at least one dose of study medication (T₃) in combination with sertraline and returned for clinical assessments were included in the intent to treat (ITT) sample and in the final data analysis. Categorical variables were compared with contingency tables with χ^2 and Fishers exact statistics. Continuous variables were compared with t tests and ANOVA as appropriate. Logistic regression modeling was utilized to investigate factors impacting remission status. All statistical significance was defined as two-tailed $\alpha < 0.05$.

Results: A total of 153 subjects are included in the modified ITT analysis and there were 108 subjects (70.6%) that completed all of the study visits. Overall 70 (45.7%) subjects met remission criteria at final study visit. Mean (\pm SD) HRSD-21 score at baseline for all subjects was 22.8 (\pm 4.4), 22.2 (\pm 4) for those who achieved remission, and 23.3 (\pm 4.7) for those who did not. There were 51 subjects (33.3%) who were antidepressant naïve and 102 (66.6%) with at least one previous episode of medication exposure. Of the treatment naïve subjects, 32 of 51 (62.8%) achieved remission, while 38 of 102 (37.3%) of the previously treated individuals reached this goal ($p < 0.003$; two-tailed). Subjects who remitted (15.7 yrs (\pm 1.6)) had more years of education on average than those who did not (14.7 yrs (\pm 2.0)) ($F = 9.6$; $df = 1, 151$; $p < 0.002$). Baseline depression severity, number of previous depressive episodes, age, gender or race did not impact remission status. There was no difference in final visit sertraline dose between those who remitted (146.4 mg (\pm 44.5)) and those who did not (143.4 (\pm 52.2)). A logistic regression model was calculated that included study completion, previous treatment, gender, age, race, years education, number past depressive episodes and baseline HRSD-21 score as regressors on remission status ($\chi^2 = 38.9$; $df = 8$; $p < 0.0001$). Those factors with a significant effect were study completion (OR = 4; $p < 0.003$), no previous treatment (OR = 4.9; $p < 0.0005$) and years of education (OR = 1.3 per year education; $p < 0.02$).

Discussion: Antidepressant naïve patients with major depressive disorder were much more likely to reach remission with SSRI than those with previous episodes of treatment. The implication of this finding is that the window of maximal opportunity to achieve remission and hence long term recovery for patients with depression is at the very beginning of treatment. Non-psychiatrist physicians generally initiate treatment for depression for the majority of patients, so educational efforts should be focused on increasing awareness of this outcome goal and methods to achieve it among these practitioners.

Disclosure: S. Garlow: Part 1; Eli Lilly Co. P. Ninan: Pfizer. Part 5; Pfizer. C. Nemerooff: Part 1; AstraZeneca, Forest, Quintiles, Janssen/

Ortho-McNeil, PharmaNeuroboost, Mt Cook Pharmaceuticals, Concept, Revaax, NovaDel Pharma, CsNeRx, AFSP, George West Mental Health Foundation, NARSAD.

52. Double-Blind, Placebo-Controlled Efficacy and Safety Study of Lisdexamfetamine Dimesylate in Adolescents with Attention-Deficit/Hyperactivity Disorder

Maria Gasior*, Robert Findling, Ann Childress, Andrew Cutler, Mohamed Hamdani, M. Celeste Ferreira-Cornwell

Shire Development Inc., Wayne, PA

Background: Attention-deficit/hyperactivity disorder (ADHD), a neurobehavioral disorder that begins in childhood, is a chronic condition that often persists into adolescence. The prevalence of ADHD worldwide is estimated to be approximately 6.5% in school-aged children; estimates of prevalence range from approximately 3% to 10% in adolescents. Similar to children with ADHD, adolescents may have inattention, impulsivity, and continue to exhibit functional impairments in multiple life settings. However, adolescents may have decreased degrees of hyperactivity relative to younger children. For individuals with ADHD, psychostimulants are considered a first-line treatment. Long-duration stimulant preparations for adolescents may be helpful, as they alleviate the need to take medications throughout the day and help maintain the adolescent's privacy by avoiding trips to the nurse's office during the school day. Lisdexamfetamine dimesylate (LDX, Vyvanse®, Shire US Inc.) is a long-acting prodrug stimulant indicated for ADHD in children (6-12 years) and in adults, but not adolescents (13-17 years). LDX is endogenously converted to d-amphetamine. Placebo-controlled studies that focus on adolescents with ADHD are limited, and no study has previously been conducted with LDX in adolescents. This study examined the efficacy and safety of LDX vs placebo in adolescents with ADHD.

Methods: Eligible subjects (13-17 years) with at least moderately symptomatic ADHD (ADHD Rating Scale IV: Clinician Version [ADHD-RS-IV] score ≥ 28) were randomized to placebo or LDX (30, 50, or 70 mg/d) with forced-dose titration in a 4-week, double-blind study. Primary and secondary efficacy measures were the ADHD-RS-IV, Clinical Global Impressions-Improvement (CGI-I) scale, and Youth Quality of Life-Research Version (YQOL-R). Safety assessments included treatment-emergent adverse events (TEAEs), vital signs, laboratory findings, physical exam, and electrocardiogram (ECG).

Results: Overall, 314 subjects were randomized, 309 included in efficacy analyses, and 49 withdrew (11 due to TEAEs). At endpoint, changes in ADHD-RS-IV total score were significantly greater for each LDX dose vs placebo; least squares mean (SE) change from baseline was -18.3 (1.25), -21.1 (1.28), and -20.7 (1.25) for 30, 50, and 70 mg/d LDX, respectively, and -12.8 (1.25) for placebo ($P < .006$ vs placebo for each). Significant differences in ADHD-RS-IV total and both subscale scores relative to placebo were observed in each LDX group beginning at week 1 and at every subsequent week throughout the study ($P \leq .0307$). The percentage of subjects rated very much or much improved at endpoint as measured by CGI-I was significantly greater for LDX (all doses) than for placebo (69.1% vs 39.5% [$P < .0001$], respectively). LDX vs placebo did not show statistically significant changes from baseline at endpoint in YQOL-R total score and domain scores. The most frequently reported LDX TEAEs ($\geq 5\%$) included decreased appetite, headache, insomnia, weight decrease, and irritability. There were small mean increases in pulse and systolic and diastolic blood pressure with LDX. There were no clinically meaningful trends in laboratory or ECG measurements noted with LDX.

Discussion: LDX treatment (30, 50, 70 mg/d) was effective vs placebo as measured by change from baseline at endpoint in the ADHD-RS-IV total score. Efficacy of LDX vs placebo was demonstrated beginning at week 1 and continuing to the week-4 endpoint on ADHD-RS-IV total and subscale scores. More LDX subjects were rated as improved vs placebo by the CGI-I, at every study week and endpoint. LDX

demonstrated a safety profile consistent with previous LDX studies in children or adults. Supported by funding from Shire Development Inc.

Disclosure: M. Gasior: Part 1; Shire. Part 2; Shire. Part 3; Shire. Part 4; Shire. Part 5; Shire. R. Findling: Part 1; Abbott, Addrenex, AZ, Bioavail, BMS, Forest, GSK, J&J, KemPharm Lilly, Lundbeck, Neuropharm, Novartis, Noven, Organon, Otsuka, Pfizer, SA, Schering, Sepracor, Shire, Solvay, Supernus, Validus, Wyeth. Part 2; n/a. Part 3; n/a. Part 4; Abbott, Addrenex, AZ, BMS, Forest, J&J, Kempharm Lilly, Neuropharm, Otsuka, Pfizer, Shire, Supernus, Wyeth. Part 5; n/a. A. Childress: Part 1; Abbott, Bristol-Myers Squibb, Johnson & Johnson Pharmaceutical Research & Development, Lilly USA, NextWave, Novartis, Ortho-McNeil Janssen Scientific Affairs, Shire, Somerset. Part 2; GlaxoSmithKline, Novartis, Shire. Part 3; GlaxoSmithKline, Shire. Part 4; Abbott, Bristol-Myers Squibb, Johnson & Johnson Pharmaceutical Research & Development, Lilly USA, NextWave, Novartis, Ortho-McNeil Janssen Scientific Affairs, Shire, Somerset. Part 5; n/a. A. Cutler: Part 1; Abbott, Addrenex, AZ, BMS, Cephalon, GSK, Janssen, Jazz, J&J, Lilly, McNeil, Memory, Merck, NEI, Novartis, OMN, Otsuka, Pfizer, Sanofi, Sepracor, Shire, Shionogi, Solvay, Supernus, Targacept. Part 2; AstraZeneca, Merck, Novartis, Shire. Part 3; n/a. Part 4; Abbott, Addrenex, AZ, BMS, Cephalon, GSK, Janssen, Jazz, J&J, Lilly, McNeil, Memory, Merck, Novartis, OMN, Otsuka, Pfizer, Sanofi, Sepracor, Shire, Shionogi, Solvay, Supernus, Targacept. Part 5; n/a. M. Hamdani: Part 1; Shire. Part 2; Shire. Part 3; Shire. Part 4; Shire. Part 5; Shire. M. Ferreira-Cornwell: Part 1; Shire. Part 2; n/a. Part 3; n/a. Part 4; Shire. Part 5; Shire.

53. Results of the Validation of Everyday Real World Outcomes (VALERO) Study: Validation of 6 Real World Rating Scales for their Relationship with Neurocognitive and Functional Ability

Philip Harvey*, Elizabeth Twamley, Lea Vella, Thomas Patterson, Robert Heaton

University of Miami Miller School of Medicine, Miami, FL

Background: Cognitive deficits are associated with disability in people with schizophrenia. However, several recent studies have found very minimal relationships between ratings of real-world functioning and cognitive performance. These studies typically relied on patient self-report and there is little information about which rating method or informant provides the best information. It is also not clear which rating strategies (consensus, informant only, etc.) would provide the best convergence between real world functioning and ability measures. Thus, the Validation of everyday real world outcomes (VALERO) study was conducted, which evaluated 6 real-world functional rating scales chosen as the most suitable of the current candidates by a RAND panel and examined the correlations between scores on these rating scales and performance-based measures of cognitive functioning and the ability to perform everyday activities.

Methods: One hundred and ninety-eight (198) people with schizophrenia were tested with the MATRICS Consensus cognitive Battery (MCCB) and performed the UCSD performance-based skills assessment-B (UPSA-B), and the advanced finances subtest from the Everyday functioning battery (EFB). They and an informant (Friend, relative, or case manager) also reported their functioning on 6 real world functional status ratings scales ; The Social Behavior Schedule (SBS), The Social Adjustment Scale (SAS), the Heinrichs Carpenter Quality of Life Scale (QLS), the Specific Levels of Functioning (SLOF), the Independent Living skills Inventory (ILSS), and the Life Skills Profile (LSP). Best judgment ratings were generated by an interview who conducted both interviews and these ratings were used for the correlational analyses.

Results: HLM analyses were used to construct an ability latent trait from the three performance-based measures and canonical correlation analysis was used to relate all 6 functional status rating scales to the latent trait derived from the three performance-based measures. The overall model fit was quite good: $\chi^2 = 78.100$, degrees of freedom = 56, p-value = .027, and RMSEA = .078, with 41% variance shared between

the ability latent trait and the 6 rating scales. The rating scales were systematically deleted one by one from the model and the final model with two rating scales, the LSP and the SLOF, fit the data: $\chi^2 = 32.059$, df = 24, p = .126, RMSEA = .072. A regression analysis found that the LSP did not add any variance to the prediction of the ability latent trait above and beyond the SLOF.

Discussion: Systematic assessments of real world functioning are quite strongly related to performance on ability measures such as the MCCB and UPSA-B. Of the six rating scales selected as most suitable by the VALERO RAND panel, the Specific Levels of Functioning was best in this study.

Disclosure: P. Harvey: Part 1; Eli Lilly, Abbott Labs, Shire Pharma, Johnson and Johnson, Solvay Pharma, Cypress BioScience, Wyeth Pharma, Merck and Company, Teva Pharma. Part 2; Sepracor Pharma. Part 4; Astra Zeneca. E. Twamley: None. L. Vella: None. T. Patterson: None. R. Heaton: None.

54. Ondansetron Augmentation in Treatment-Resistant OCD (TR-OCD): Relapse in Y-BOCS Symptoms Following Discontinuation of Ondansetron

Stefano Pallanti, Silvia Bernardi, Eric Hollander*

Montefiore Med Ctr of Albert Einstein COM, Bronx, NY

Background: About 40% of OCD patients do not respond to first line serotonin reuptake inhibitor (SRI) treatment. For one third of those, antipsychotic augmentation has been reported to improve the rate of response, possibly by blocking the effects of striatal dopamine release, resulting in improvement in habits. By down regulating dopamine release in the cortico-mesolimbic pathway of the brain, the 5-HT₃ antagonist ondansetron has the potential to augment therapeutic benefits of SRIs in OCD patients who fail to adequately respond to SRI monotherapy. The objective of the study was to investigate whether ondansetron would be effective as an augmentation therapy to SRI monotherapy for treatment-resistant OCD, and if effective, whether symptoms would recur after the drug was discontinued. Demonstration of effectiveness during the treatment period, and relapse of symptoms after discontinuation, would both signal a potential therapeutic efficacy of ondansetron for TR-OCD. Eventually, if proven safe and effective, low-dose ondansetron might replace antipsychotic augmentation use, thereby eliminating the safety and tolerability issues of antipsychotic augmentation therapy.

Methods: Twenty-one patients with a DSM-IV diagnosis of treatment-resistant OCD, under stable treatment with SRIs approved by the FDA for OCD therapy, received 12 weeks of single-blind ondansetron augmentation initiated at a dose of 0.25 mg twice daily for 2 weeks, and titrated to 0.5 mg twice daily for 10 weeks. Patients were rated every two weeks using the Yale Brown Obsessive Compulsive Scale (YBOCS) and Clinical Global Impressions Scale (CGI). Treatment-resistant patients were defined as having completed an adequate trial of SRIs at a moderate to high dose for at least 12 weeks and still having a YBOCS severity of > 24 , and CGI-Severity of ≥ 4 . Treatment response was defined as an additional 25% reduction in YBOCS score from YBOCS score at the initiation of ondansetron augmentation, an end of treatment (EOT) period YBOCS of ≤ 24 , and CGI-Improvement (CGI-I) of ≤ 2 . Ondansetron was discontinued after 12 weeks and patients were followed for another 4 weeks for relapse in YBOCS symptoms.

Results: Twelve of the 21 (57%) patients in the study experienced a treatment response in 12 weeks or less based on 25% improvement in YBOCS, EOT YBOCS score < 24 , and CGI-I = 1-2. The average reduction in YBOCS-rated symptoms of the whole group was 26.3%. The average reduction in CGI scores for the whole group was 46%. Responders had a 44% reduction in YBOCS and 65% reduction in CGI-I from baseline. Non-responders showed marginal (2.9%) improvement in YBOCS scores. During the discontinuation phase the YBOCS symptoms worsened by 14.6% in all patients, and 38.3% in responders. Treatment was well tolerated.

Discussion: These preliminary efficacy, safety, and relapse data suggest that patients who do not adequately respond to SRI treatment may benefit from augmentation with a low dose of ondansetron. Results of this study need to be confirmed in longer duration and larger placebo-controlled clinical trials. If replicated, this may provide an alternative to the use of anti-psychotic augmentation, and provide a more favorable adverse event profile.

Disclosure: S. Pallanti: Part 1; Transcept. Part 4; Transcept. S. Bernardi: None. E. Hollander: Part 2; Transcept. Part 4; Transcept.

55. Efficacy of Asenapine for Schizophrenia: Comparison With Placebo and Comparative Efficacy of All Atypical Antipsychotics Using All Available Head-to-Head Randomized Trials Using Meta-Analytical Techniques

Armin Szegedi*, Pierre Verweij, Wilbert Van Duijnhoven, Mary Mackle, Pilar Cazorla, Craig Karson, Hein Fennema

Merck, Rahway, NJ

Background: Asenapine is an atypical antipsychotic (AAP) indicated in the US in adults for treatment of acute schizophrenia. To characterize the efficacy of asenapine in this indication, data from all placebo-controlled 6-week trials were analyzed. The efficacy of both asenapine and active controls versus placebo was confirmed in 2 studies, but efficacy was less than that historically observed when compared with other placebo-controlled AAP trials due to a large placebo response. To characterize the relative efficacy of asenapine, all randomized head-to-head comparisons of asenapine to AAPs and published randomized head-to-head comparisons of AAPs in the treatment of schizophrenia were analyzed. This analysis provides a comparison of asenapine to all AAPs, including those for which no direct comparisons are available. **Methods:** The efficacy of asenapine versus placebo was analyzed using 4 placebo-controlled trials that included treatment arms in the effective dose range (5 to 10 mg BID asenapine); dosage groups were pooled in the analyses. The primary efficacy outcome, Positive and Negative Syndrome Scale (PANSS) total score change from baseline at week 6, was assessed using last observation carried forward (LOCF) to impute missing data (primary analysis) and a mixed model for repeated measurements (MMRM; sensitivity analysis). PANSS responders (patients with a decrease of $\geq 30\%$ from baseline at endpoint) were analyzed to illustrate clinical relevance. All meta-analyses used individual patient data. A meta-regression in which asenapine data was added to data described by Leucht et al (*Mol Psychiatry* 2008; 14: 429-447) investigated the time course of the decline in observed treatment effect in schizophrenia for AAPs versus placebo. A network meta-analysis was conducted to estimate relative efficacy among all AAPs based on randomized head-to-head comparisons. This analysis included 74 studies described by Leucht et al (*Am J Psychiatry* 2009; 166:152-166) and was updated with results from all AAP-controlled 6-week asenapine trials and a 52-week olanzapine-controlled asenapine trial. The network meta-analysis used a two-stage approach: (1) a random-effects meta-analysis established the relative efficacy among all AAPs based on PANSS total score change from baseline; (2) efficacy estimates were then included in a regression analysis that was weighted using the precision of each meta-analysis. This analysis provided maximum-likelihood-based estimates of comparative efficacy of all AAPs on PANSS total score.

Results: Based on all randomized placebo-controlled studies, the change in PANSS total score at week 6 was significantly greater with asenapine than placebo (LOCF: -3.7 [95% CI, -5.9 to -1.5], $P = 0.001$; MMRM: -4.1 [95% CI, -6.5 to -1.6], $P = 0.001$). The efficacy of asenapine relative to placebo was comparable to that of the combined active controls used in the studies (LOCF: -4.1 [95% CI, -6.5 to -1.7], $P = 0.001$; MMRM: -4.6 [95% CI, -7.3 to -1.9], $P = 0.001$). Analysis of PANSS responder rates illustrated the clinical relevance of these effects, with an overall odds ratio of 1.9 for asenapine over placebo (95% CI: 1.4 to 2.6, $P < 0.001$) and a corresponding number needed to treat (NNT) of 10.2. The effects of asenapine relative to placebo were comparable to

those of the combined active controls (odds ratio = 1.7 [95% CI: 1.2 to 2.4, $P = 0.002$]; NNT = 12.0). Applying meta-regression on the observed treatment effects of AAPs versus placebo confirmed a declining trend ($P < 0.001$), with lesser effects observed in more recent years. Using network meta-analysis on head-to-head comparisons, the efficacy of asenapine was found to be comparable to other AAPs; the estimated PANSS total score difference between asenapine and other AAPs ranged from 3.9 points (95% CI, 0.3 to 7.4) better than ziprasidone to 2.9 points (95% CI, -5.9 to 0.1) worse than olanzapine. Risperidone and amisulpride slightly outperformed asenapine (1.0 and 0.5 points, respectively) but asenapine performed better than aripiprazole (1.6 points), quetiapine (1.1 points), sertindole (1.0 points), and clozapine (0.3 points).

Discussion: These meta-analyses demonstrate the superiority of asenapine over placebo in the treatment of acute schizophrenia. The efficacy of asenapine, based on mean PANSS total score change and PANSS responder rates, was comparable to that of combined active controls used in the same studies. The network meta-analysis using all available randomized head-to-head comparisons suggested that the efficacy of asenapine is comparable to a group of established AAPs in treating acute schizophrenia.

Disclosure: A. Szegedi: Part 5; Merck. P. Verweij: Merck Sharp & Dohme. W. van Duijnhoven: Merck Sharp & Dohme. M. Mackle: Merck. P. Cazorla: Merck. C. Karson: Merck. H. Fennema: Merck Sharp & Dohme.

56. Diagnostic Sensitivity and Specificity for Alzheimer's Disease Dementia Based on ERK Phosphorylation Responses of Cultured Fibroblasts

C. Anthony Altar*, Jun Chen, Safdar Jawaad, Tapan Khan, Daniel L. Alkon

Blanchette Rockefeller Neuroscience Institute, Rockville, MD

Background: A variety of changes in the Alzheimer's disease (AD) brain, such as elevations in secreted β -amyloid, decreased PKC (Tsang et al, 2007; Wang et al, 1994; Battaini et al, 1999), and increases in phosphorylated extracellular signal-regulated kinase (p-ERK1/2; Veeranna et al, 2004; Pei et al, 2002; Ferrer et al, 2001) are also present in skin fibroblasts cultured from AD patients. Fibroblasts from AD cases secrete more β -amyloid (Scheuner et al, 1996; Johnston et al, 1994) and contain less PKC protein and activity (Govoni et al, 1996; Bruel et al, 1991; Van Huynh et al, 1989). Amyloid exposure induces AD features in fibroblasts from non-AD cases (Etcheberriigaray et al 1994, 1996), including an increase in p-ERK1 relative to p-ERK2 protein levels. This relative increase in p-ERK1 can be reversed by PKC epsilon activation (Khan et al, 2009). An increase in the p-ERK1/p-ERK2 ratio is also obtained in bradykinin (BK)-stimulated AD fibroblasts, whereas a decrease in this ratio is characteristic of age-matched control cases (Khan & Alkon, 2006, 2008). This ratio change in response to BK, termed the AD Index, was evaluated here in an independent cohort of AD and non-AD cases, and analyzed as a function of family history or post-mortem confirmation of brain amyloid plaques in some of the AD cases.

Methods: Human skin fibroblasts from 29 AD cases and 28 non-demented, non-autopsy confirmed, age-matched controls (AC) were obtained from the Coriell Institute of Medical Research. The mean age of the 18 male and 9 female AD cases was 64.7 ± 10 years, versus 60 ± 9.8 years for the 16 male and 12 female controls. Fibroblasts were grown to 90% confluence, serum-starved for 16 h, and then exposed for 10 min to vehicle or 10 nM BK, each in DMEM with 10% FBS. The cells were lysed, the total protein content was measured, and processed for quantitative p-ERK1/2 immunostaining based on proteins transferred from SDS-PAGE gels.

Results: Positive AD Index values predictive of AD status were present in 90% of the 29 AD cases and in 100% of the 9 autopsy-confirmed AD cases and the 6 familial AD cases. Negative AD Index values predictive of control (AC) status were found in 71% of the 28 control cases,

resulting in a highly significant AD Index difference between the AD and AC groups ($p < 0.001$). The overall accuracy of this method was 81%, and the distribution of values was highly reminiscent of prior reports (Khan & Alkon, 2006, 2008).

Discussion: This study replicates prior findings (Khan and Alkon, 2006) and those of an independent cohort of patients from 15 hospitals included in the CAVEAT study (Khan and Alkon, 2008). The method was fully sensitive for autopsy-confirmed AD cases, also as reported previously. The degree to which the AC cases with positive, AD-like AD Index values may have had latent AD status at the time of biopsy is unknown. The AD Index may provide a method for distinguishing individuals who develop AD dementia from those who do not. Clinical trials are being pursued to evaluate this and other diagnostic measures. This work was conducted in collaboration with Alere, Inc., Waltham, MA 02453.

Disclosure: C. Altar: None. J. Chen: None. S. Jawaid: None. T. Khan: None. D. Alkon: None.

57. Oxytocin Selectively Improves Empathic Accuracy in Adults Endorsing Traits of Autism

Jennifer Bartz*, Jamil Zaki, Niall Bolger, Eric Hollander, Natasha Ludwig, Alexander Kolevzon, Kevin Ochsner

Mount Sinai School of Medicine, New York, NY

Background: Oxytocin (OT) regulates prosocial behavior and aspects of social cognition in animals and recent data suggests that it may have similar functions in humans. For example, OT was shown to facilitate trust behavior and mental state attribution accuracy (e.g., Kosfeld et al 2005; Domes et al 2007). Such findings have led some to conclude that OT has broad positive effects on social perception and function. Careful inspection of the literature, however, suggests that OT's effects on human social cognition are neither simple nor obvious. Rather than having broad positive effects, OT may alter specific motivational or cognitive states (that, e.g., make social cues more salient), which in turn could improve social cognitive performance for some individuals, but not others. Accordingly, the effects of OT should be most pronounced for individuals who, at baseline, are less socially proficient. This hypothesis is consistent with broader interactionist views emphasizing that individual differences in competencies interact with situational variables to determine behavior.

Methods: Twenty-seven healthy men participated in this double-blind, placebo-controlled, cross-over challenge and received intranasal OT/placebo on two occasions (3-5 weeks apart). Forty-five minutes later participants completed an Empathetic Accuracy task that naturalistically measures social cognitive abilities. Specifically, participants watched and listened to short videos of targets discussing positive or negative autobiographical events and provided continuous ratings of how positive/negative the target was feeling. These ratings were compared to actual target ratings of target affect. Empathetic accuracy was measured as the time-series correlation between perceiver and target ratings. Individual differences in social proficiency were assessed at baseline with the Autism Spectrum Quotient (AQ).

Results: In contrast to the popular view, mixed-model analyses showed no main effect of OT on empathetic accuracy ($t < 1$). However, consistent with the interactionist hypothesis, there was a significant effect of AQ ($b = -0.11$, $t(79) = -2.77$, $p < .01$), and a significant Drug X AQ interaction ($b = 0.11$, $t(232) = -2.01$, $p < .05$) predicting empathetic accuracy performance. Participants endorsing few autism traits performed well on the empathetic accuracy task following placebo and maintained this performance level following OT. By contrast, participants endorsing many autism traits performed more poorly following placebo than their lower AQ counterparts, but, critically, they showed the greatest improvement from OT; indeed, following OT, their performance was indistinguishable from their lower AQ counterparts.

Discussion: OT is not a universal prosocial enhancer that can render all people social cognitive experts. Rather OT appears to play a more nuanced role in social cognition, possibly increasing the salience of

social cues and, therefore, is only helpful for those who are less sensitive to social cues at baseline like our high AQ scorers. Although our participants were healthy adults, the fact that OT selectively improved empathetic accuracy for participants endorsing autism traits highlights the promise OT holds for treating real-life social cognition in autism.

Disclosure: J. Bartz: None. J. Zaki: None. N. Bolger: None. E. Hollander: Part 1; Applied for a patent for oxytocin and autism. N. Ludwig: None. A. Kolevzon: None. K. Ochsner: None.

58. Persistent Deficits in Euthymic, MDD Patients in an Attentional Rubbernecking Task

Jason Chen, Steven Most, Barbara Ruf, Kathleen Maloney, Aybala Saricicek, David Matuskey, Zubin Bhagwagar*

Bristol-Myers Squibb, Wallingford, CT

Background: A number of neurocognitive processes have been shown to be dysfunctional in acutely depressed patients with major depressive disorder (MDD). However it is unclear if these deficits persist into full recovery from MDD, without the confound of medication. "Attentional rubbernecking" is a phenomenon in which task-irrelevant, emotional stimuli spontaneously grab attention, impairing visual awareness of subsequent targets. In previous studies, healthy volunteers were less able to detect targets that followed soon after a task-irrelevant negative emotional distracter that had been inserted into the stream, relative to those following an emotionally neutral distracter. We hypothesized that remitted depressed participants would have enduring, persistent deficits resulting in more impaired target detection following emotionally.

Methods: Unmedicated, remitted, early-onset, familial, recurrent depressed ($N = 24$) and healthy volunteers ($N = 32$) who did not differ in age, gender, or verbal IQ were administered the attentional rubbernecking task. Participants were asked to search through rapidly presented streams (100-ms/item) of upright landscape photos and indicate whether a target landscape had been rotated 90-degrees right or left. Targets appeared either two (lag-2) or eight (lag-8) pictures after a neutral or negative emotional stimulus. Lag-2 data were analyzed for differences between groups while lag 8 data were used as a control condition and showed no group differences. We also collected personality measures using the NEO Personality Inventory and the State-Trait Anxiety Inventory.

Results: Controlling for performance in the emotionally neutral condition as well as age and gender via an ANCOVA analysis, we found that remitted depressed individuals exhibited significantly worse target detection following a negative emotional distracter compared with healthy controls ($F(1, 58) = 2.18$, $p = 0.038$). Lag-2 negative accuracy had a significant positive correlation with extraversion ($r(27) = .539$, $p = .003$) and a negative correlation with trait anxiety ($r(27) = -.480$, $p = .008$) in healthy volunteers but not in the patient group. As expected, there were no between group differences for the lag-8 condition ($p = 0.870$).

Discussion: Decreased performance on the attentional rubbernecking task as compared with healthy volunteers suggests a specific, continuing deficit in neural function which may predict trait vulnerability for major depressive disorder. It is possible that these factors may be modulated by personality variables.

Disclosure: J. Chen: None. S. Most: None. B. Ruf: None. K. Maloney: None. A. Saricicek: None. D. Matuskey: None. Z. Bhagwagar: Part 5; Bristol-Myers Squibb.

59. Evidence-Based Science: Developing Causal Models for Neuropsychopharmacology Research

Robert Bilder*, D. Stott Parker, Andrew Howe, Fred W. Sabb

UCLA Semel Institute, Los Angeles, CA

Background: As the complexity of integrative neuroscience models increases, spanning multiple biological and behavioral scales from

genome to syndrome, the likelihood that any individual can successfully assemble a comprehensive hypothesis decreases. While progress has been made in the development of knowledgebases for genomic, molecular expression, and some signaling pathway evidence, higher phenotypic levels (at neural system, cognitive, symptomatic and syndromal) are not yet well represented in these information architectures. Further, most repositories of relevant information are either minimally structured collections of tagged free text annotation (e.g., Online Mendelian Inheritance in Man or OMIM) or structured databases with little flexibility to support complex relations (e.g., Entrez Gene). Recent studies also make it clear that we are likely to be deluged with a multitude of genetic signals that are only weakly associated with complex phenotypes, increasing the need for models that can support mechanistic modeling of relations across multiple biological scales to help focus research on the most informative causal paths. Given that no individual is likely to possess detailed knowledge at all relevant biological scales, there is a need to develop collaborative frameworks so that experts can contribute domain-specific information. A collaborative framework further is capable of supporting more rapid growth and realization of a useful knowledge resource.

Methods: We are developing a framework to represent mechanistic neuroscience theories under the aegis of the Hypothesis Web project (RL1LM009833) in the Consortium for Neuropsychiatric Phenomics at UCLA. This framework relies extensively on graphical representation of concepts and their inter-relations, offering multiple advantages in visualization (including panning and zooming on sub-hypotheses), connecting graphs that may originally have represented multiple discrete chains of evidence into more comprehensive hypotheses, and permitting ultimately the application of methods for automated meta-analyses, and assessing causality by performing “graph surgery,” and applying the tools of probability calculus and structural equation modeling. The key elements of the higher level representations include syndromes which are operationally defined by the algorithmic conjunction and disjunction of symptoms, which in turn can be related to cognitive concepts, and to neural system models. Each of the higher level symptom and cognitive concepts is linked explicitly to measurement models, enabling the formal representation of experimental findings at the group or study level (suitable for meta-analysis) and also from individual cases (suitable for structural equation modeling). The neural system models are represented as directed acyclic graphs, containing as their primary elements canonical cells and their projections to targets. These cellular units use the lexica of other knowledgebases (e.g., Gene Ontologies) enabling the neural system models to be linked to existing resources for representation of information about molecular entities, genomics, proteomics, signaling pathways and other biological processes. Confidence in hypotheses may be expressed by the quality (and quantity) of evidence that supports the hypothesis. The minimal (Level 1) specification of a hypothesis involves selecting specific concept nodes and their putative relations to each other. Level 2 specification of a hypothesis involves annotating the conceptual hypothesis with evidence (using free text) from specific published literature. Level 3 specification of a hypothesis involves adding quantitative annotation that represents each concept with empirical experimental observations, thus creating a parallel structural model for each link in the hypothesis.

Results: As a proof of concept, we have created a draft representation of “working memory” hypotheses that were the focus of a recent NIMH workshop addressing Strategic Plan section 1.4 (i.e., the Research Domains Criteria (RDoC) initiative). These hypotheses include syndrome, symptom, cognitive, neural system, cellular, protein, molecular, and genetic components.

Discussion: It is hoped that this framework can help advance the formalization of hypotheses about neural system functions, their molecular bases, and their manifold higher phenotypic expressions, ultimately forging an empirical evidence-based system for developing new experimental and therapeutic strategies. Supported by NIH Roadmap Initiative grants UL1DE019580, RL1LM009833.

Disclosure: R. Bilder: Part 1; CHDI, Cypress Bioscience, Dainippon Sumitomo, Hoffman-LaRoche, Johnson & Johnson, Merck, Pfizer Inc.. Part 2; Johnson & Johnson. D. Parker: None. A. Howe: None. F. Sabb: None.

60. Expectation and Temperament Moderate Amygdala and Prefrontal Cortex Responses to Fear Faces

Jacqueline Clauss, Ronald Cowan, Jennifer Blackford*

Vanderbilt University School of Medicine, Nashville, TN

Background: Inhibited temperament is characterized by a chronic tendency to avoid novelty, and is associated with increased risk for anxiety. Neuroimaging studies have demonstrated that an inhibited temperament is associated with increased amygdalar blood-oxygenation-dependent-level (BOLD) response to novel faces that were not expected; however, the effects of variations in expectancy remain unknown. Expectation plays a critical role in information processing, with past and current information constantly used to predict future events. Given the key role of the response to novelty in inhibited temperament, one would expect inhibited individuals would also be affected by variation in expectancy.

Methods: Using functional magnetic resonance imaging (fMRI), we studied BOLD response to fear faces that were either expected or unexpected in 42 adults with an inhibited or an uninhibited temperament. Expectancy was manipulated between-subjects so that within each temperament group, half of the subjects were expecting to see the fearful faces and the other half were not. Within the amygdala region of interest, clusters showing a significant Temperament Group X Expectancy interaction were identified using SPM5 (cluster-based threshold correction, $p < .05$, cluster size > 11 voxels). Whole brain analyses were also performed using SPM5 to identify novel brain regions showing a Temperament Group x Expectancy interaction. Average percent signal change values were extracted from the significant clusters using MarsBar and post-hoc analyses of variance were performed.

Results: The temperament groups differed significantly in bilateral amygdala BOLD responses to expected compared to unexpected fear faces (both $p < .05$, corrected). When expecting to see fear faces, individual with an inhibited temperament had greater amygdala but less prefrontal cortex BOLD response in the dorsal anterior cingulate cortex (dACC) and dorsolateral prefrontal cortex (dlPFC) compared to the group not expecting to see fear faces. In contrast, those with an uninhibited temperament had a smaller amygdala response, but larger dACC and dlPFC BOLD responses when expecting to see fearful faces.

Discussion: These findings demonstrate temperament differences in expectancy effects and provide preliminary evidence for the dACC and dlPFC as neural substrates mediating differences in inhibited temperament. Enhanced amygdala sensitivity coupled with weak inhibitory control from the prefrontal cortex, may form a neural circuit supporting behaviors characteristic of inhibited temperament and risk for anxiety.

Disclosure: J. Clauss: None. R. Cowan: Part 1; Novo Nordisk, Shire Pharmaceuticals, SWMF Life Science Fund. Part 2; Novo Nordisk, Shire Pharmaceuticals. Part 3; Novo Nordisk, Shire Pharmaceuticals. Part 4; Novo Nordisk, Shire Pharmaceuticals. J. Blackford: None.

61. Fast Left Prefrontal rTMS Acutely Suppresses Analgesic Effects of Perceived Controllability on the Emotional Component of Pain Experience

Jeffrey Borckardt*, Scott Reeves, Heather Frohman, Alok Madan, Mark P Jensen, David Patterson, Mark George

Medical University of South Carolina, Charleston, SC

Background: The prefrontal cortex may be a promising target for transcranial magnetic stimulation (TMS) in the management of depression and pain. It is not exactly clear how prefrontal TMS affects

mood or pain perception, but several animal studies suggest that this area of the brain may be an important part of a circuit of 'perceived controllability' regarding pain, mood, stress and learned helplessness. This is important, especially in chronic pain, as perceived controllability is associated with decreased pain and improved functioning. The present controlled, laboratory study explored the immediate effects of fast left prefrontal TMS on the analgesic benefits of perceived pain controllability.

Methods: Twenty-four healthy volunteers underwent a laboratory pain task designed to manipulate perception of pain-controllability. During each pain-controllability trial, fast (10 Hz at 110% of MT) left prefrontal TMS was delivered during the time window wherein the analgesic benefits of perceived control were believed to be operative. Participants rated pain stimuli intensity and unpleasantness using visual analogue scales.

Results: There was a significant main effect of perceived-pain-controllability on pain intensity ($F(1,165) = 7.33$, $p = .008$) and pain unpleasantness ($F(1,309) = 5.23$, $p = .023$) such that thermal stimulation delivered during perceived control condition were rated less painful in both groups. There was a significant TMS-condition (real versus sham) by perceived-control interaction on pain unpleasantness ratings ($F(1,309) = 6.55$, $p = .011$). Real TMS, compared to sham, suppressed the analgesic benefits of perceived-control on the emotional dimension of pain.

Discussion: Findings suggest that, at least acutely, fast left prefrontal TMS may interrupt the perceived-controllability effect on the emotional dimension of pain experience. Prefrontal TMS may produce analgesic and mood regulatory effects by acting through a cortical 'perceived control' circuit regulating limbic and brainstem areas of the pain circuit.

Disclosure: J. Borckardt: Part 4; Covidien through The Obesity Society. S. Reeves: None. H. Frohman: None. A. Madan: Covidien. M. Jensen: None. D. Patterson: None. M. George: Part 1; Brainsonix, Brainsway, Neuronetics, Neostim, Neosync, PureTech Ventures, Force Protection. Part 4; Brainsway, Neuronetics, Cyberonics, St. Jude Biomedical.

62. Cannabis Use Disorders in Bipolar Disorder: Impact on Cognition and Clinical Outcomes

Raphael Braga*, Katherine Burdick, Pamela DeRosse, Yaniv Shaya, Anil Malhotra

The Zucker Hillside Hospital - North Shore-Long Island Jewish Health System, Glen Oaks, NY

Background: Cannabis is the most widely used illicit substance in Western countries and a growing body of evidence suggests a consistent association between its use and psychotic symptoms. Specifically, several studies have indicated that cannabis use might increase an individual's susceptibility to develop schizophrenia and other psychotic disorders; however, these findings have been somewhat controversial. Cannabis use has been shown to be very frequent among individuals with bipolar disorders, with estimated lifetime prevalence ranging from 30% to above 60%. Studies seeking to elucidate the impact of cannabis use disorders on clinical presentation and outcomes of bipolar disorder are limited and have produced inconsistent results. Cannabis use in bipolar disorder has been associated with greater treatment non-compliance, impaired psychosocial functioning, and a worsened course of illness including higher prevalence of psychosis. However, in at least one study, cannabis use has been associated with a more complete affective symptom remission in bipolar patients. The objective of the present study was to compare clinical and neuropsychological measures in individuals with bipolar disorder with a history of cannabis use disorder versus individuals with bipolar disorder without a history of cannabis use disorder.

Methods: We conducted a retrospective analysis of a large cohort ($N=200$) of patients diagnosed with bipolar I or II disorder, with either no history of a cannabis use disorder (CUD-; $N=150$) or a history of cannabis use disorder (CUD+; $N=50$) documented using the Structured Clinical Interview for the DSM-IV (SCID). We

compared the groups on demographic variables including age, sex, race, age of onset, premorbid IQ, education level and global assessment of functioning (GAF). We also compared groups on performance on a brief battery of neurocognitive tests that included Digit Span (forward and backward), Trails A and B, Verbal Fluency (letter and category), and the California Verbal Learning Test.

Results: Patient groups did not differ regarding age, age of onset, global assessment of functioning, or proportion of bipolar I/bipolar II subtypes. Compared to the CUD- group the CUD+ group had a higher proportion of men (62% vs 43.3%, $p = 0.022$), higher proportion of patients with a history of psychosis (82% vs 67.3%, $p = 0.048$), and were taking a higher mean number of total psychotropic medications (2.34 vs 1.95, $p = 0.012$). Subjects with history of cannabis use demonstrated significantly better performance on measures of attention (Digits forward; $t = 2.5$; $p = 0.01$), processing speed (Trails B; $t = 2.2$; $p = 0.03$), and working memory (Digits Backward; $t = 2.4$; $p = 0.02$).

Discussion: These findings suggest that in individuals with bipolar disorder, the history of cannabis use disorder is associated with history of psychosis and increased number of medications. Interestingly, the other important finding in our sample was the better neuropsychological performance in bipolar patients with history of cannabis use as compared to those with no history of cannabis use. These somewhat conflicting findings could indicate that the presence of cannabis use disorder in bipolar patients may be a reflection of the patients' overall higher cognitive functioning required to competently engage in social activities required for the drug use. This closely parallels findings from cannabis use and cognition in the schizophrenia literature. Notwithstanding, the retrospective design of the current study did not allow us to determine causality underlying the associations found. Future prospective studies are needed to elucidate the nature of this relationship.

Disclosure: R. Braga: None. K. Burdick: Part 1; Merck. P. DeRosse: None. Y. Shaya: None. A. Malhotra: Ely Lilly, Janssen, Venda Pharmaceuticals, Sunovion. Part 2; Merck, BMS, PGx Health.

63. Differential Modulation of Regional Brain Endocannabinoid Levels by Volitional Versus Forced Nicotine Administration in Rats

Matthew Buczynski*, Ilham Polis, Loren Parsons

The Scripps Research Institute, La Jolla, CA

Background: The brain endocannabinoid (eCB) system is now recognized for playing an important role in synaptic plasticity and is involved in homeostatic mechanisms regulating affect, motivation and cognition. It is not surprising, therefore, that brain eCB production is modulated in response to affective and motivational state and may be responsive to changes in cognitive load as well as the pharmacological effects of several classes of abused drugs. This opens the possibility that eCB function may be modulated by at least two forms of drug-induced neuroplasticity: neuroadaptations resulting from the direct pharmacological effects of addictive drugs, and the cognitive processes that are associated with active drug self-administration (SA). These different dimensions of drug-related plasticity may interact synergistically in the etiology of addiction. Nicotine is widely recognized as the causative agent leading to tobacco addiction, and both clinical and preclinical studies have demonstrated an important influence of cognitive factors (such as drug-associated conditioned cues) in the motivation for nicotine intake. Based on recent evidence implicating brain eCBs and related bioactive lipids such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) in the modulation of nicotine SA, we studied the effect of both response-contingent nicotine SA and non-contingent (NC) forced nicotine administration on levels of eCBs and related bioactive lipids in brain. The analytes evaluated include anandamide (AEA), 2-arachidonoyl glycerol (2-AG), OEA, PEA and a number of N-arachidonoyl (NA) conjugates of neurotransmitters such as dopamine (NA-DA), glutamate (NA-GLU), GABA (NA-GABA) and taurine (NA-TAU).

Methods: Three groups of rats with differing histories of nicotine exposure were prepared as follows: (1) volitional nicotine SA ($n=14$); (2) non-contingent forced nicotine exposure ($n=12$); (3) nicotine-naïve controls ($n=10$). Animals in group 1 were trained to SA nicotine in daily 2 h sessions in which intravenous infusion of 30 µg/kg nicotine reinforced lever pressing activity under a continuous reinforcement schedule paired with a 20 s cue light. Training continued until stable patterns of SA drug intake were achieved ($\pm 10\%$ variance for 3 consecutive days). Animals receiving NC nicotine administration received identical handling and were administered nicotine following a “yoked” design in which each animal was paired with a SA animal and received temporally correlated intravenous nicotine infusions paired with the cue light. Nicotine-naïve control animals were given operant sessions in which lever pressing was reinforced by intravenous saline. Animals were euthanized 1 h into the final session, and dissected for the following brain regions: nucleus accumbens (NAc), VTA, prefrontal cortex (PFC), substantia nigra (SN), dorsal striatum (DStr), amygdala (AMYG) and hippocampus (HIPP). Lipids were subsequently extracted and quantified by LC-MS/MS.

Results: As compared with levels in drug-naïve control brains, NC nicotine exposure resulted in significant increases in AEA content in mesocorticolimbic structures [VTA (129%), NAc (136%), PFC (125%)], mesostriatal structures [SN (314%), DStr (127%)] and amygdala (127%). No AEA changes in HIPP were evident. Volitional nicotine SA produced comparable AEA increases in VTA (116%) and DStr (118%) as produced by NC nicotine. However the increased AEA levels observed in the NAc and SN following NC nicotine administration were absent in animals given volitional access to nicotine. Forced nicotine exposure was associated with increased levels of NA neurotransmitters such as NA-GLU (PFC, SN, NAc), NA-TAU (PFC, DStr) and NA-GABA (PFC, DStr). However, while comparable regionally specific increases in NA-GABA were evident in nicotine SA animals, no changes in levels of NA-GLU or NA-TAU were evident relative to nicotine-naïve controls. No changes in tissue 2-AG or NA-DA were evident following either SA or NC nicotine exposure.

Discussion: Forced NC nicotine exposure is associated with robust increases in AEA and various NA-NT levels in aspects of the mesocorticolimbic and mesostriatal systems. However, volitional nicotine SA is associated with a suppression of these nicotine-induced AEA and NA-NT effects in specific regions including the NAc and SN. The relative contribution of possible stress effects (forced drug exposure) and cognitive influence (self-administration) remains to be explored. However, these findings underscore striking differences in drug-induced neuroplasticity associated with volitional versus forced drug exposure.

Disclosure: M. Buczynski: None. I. Polis: None. L. Parsons: None.

64. Low Level Visual Processing Deficits Contribute to Contour Integration Impairment in Schizophrenia

Pamela Butler*, Ilana Abeles, Pejman Sehatpour, Elisa Dias, Marina Ross, Daniel Javitt

Nathan Kline Institute, Orangeburg, NY

Background: People with schizophrenia exhibit deficits in integrating visual information. This is seen in a contour integration task in which patches of visual information have to be integrated into a whole. People with schizophrenia also show deficits in early visual processing dysfunction. This is seen in preferential impairment of the magnocellular (M) pathway/dorsal visual stream. The “frame and fill” hypothesis in controls suggests that object recognition depends on fast feed-forward magnocellular/dorsal visual stream projections to frontal areas and subsequent feedback to ventral stream object recognition areas. This study utilized event related potentials to investigate the role of this circuitry, including the role of early visual processing deficits, in visual contour integration dysfunction in schizophrenia.

Methods: People with schizophrenia and controls participated. A well-validated contour integration task was used (Kozma-Wiebe et al., Computers in Human Behavior 2006; 22:971-980). Egg-shaped contours, which pointed to the right or left, were created from isolated gabor patches. There were two difficulty conditions. In the “easy” condition, gabors were jittered 7-8 degrees. In the “difficult” condition, gabors were jittered 27-28 degrees, which makes it very difficult to see the egg-shaped contour. Participants were asked to determine whether the contours pointed to the right or left. EEG was recorded using a 68 channel Advanced Neuro Technology system. Early sensory components P1 and N1 were examined, as was the later closure negativity component N_{CL}, identified at ~250-350 ms, which indexes the ability to recognize a fragmented object.

Results: People with schizophrenia had significantly decreased P1 ($F_{1,17}=9.3$, $p=0.007$), but not N1, amplitude compared to controls indicating that initial sensory input to the dorsal stream is impaired, whereas initial input to ventral stream is relatively intact. For sensory components P1 and N1 there was no significant main effect of jitter (easy vs difficult). However, the N_{CL} component was more prominent for the easy vs. difficult jitter condition (main effect of jitter: $F_{1,17}=26.8$, $p<0.001$), confirming that N_{CL} amplitude reflects integration-related activity rather than overall stimulus energy, which is identical in the easy and high jitter conditions. There was a significant difficulty x cohort interaction ($F_{1,17}=9.9$, $p=0.006$) reflecting that people with schizophrenia, unlike controls, did not produce an enhanced N_{CL} waveform to the easy vs. difficult jitter condition. As expected, source analysis (modeled in the easy-jitter condition for controls) for the P1 showed a dorsal stream source, while the N1 and N_{CL} showed a ventral stream lateral occipital complex (LOC) source. LOC source waveforms indicate reentrant activity in this region with early sensory contributions to the generation of N_{CL}.

Discussion: These results support a circuit involving magnocellular/dorsal stream input to LOC for successful perceptual contour integration in controls. Taken together, results indicate that impaired M pathway/dorsal stream function contributes to perceptual organization deficits in schizophrenia.

Disclosure: P. Butler: Part 1; NIMH. I. Abeles: None. P. Sehatpour: None. E. Dias: None. M. Ross: None. D. Javitt: Part 1; Schering-Plough, Pfizer, AstraZeneca, NPS Pharmaceuticals, Sanofi, Solvay, Takeda, Sepracor, Lundbeck, Cypress, Merck. Part 2; Nathan Kline Institute, NYU School of Medicine, Glytech. Part 3; Glytech. Part 4; Pfizer, Jazz, Roche.

65. Impairment of Sleep Continuity Is Associated with Behavior Problems in Children of Alcoholics

Deirdre Conroy*, Ilana Hairston, Mary Heitzeg, Roseanne Armitage, Robert Hoffmann, Kirk Brower, Robert Zucker

University of Michigan, Ann Arbor, MI

Background: Sleep problems in childhood may lead to early onset of behavioral problems including early drinking. Little is known, however, about how neurophysiologic differences in sleep or circadian rhythms may predispose to and perpetuate substance-related and mood-related symptoms in children deemed at high-risk with a positive parental history of alcohol dependence and at low-risk, those without parental alcoholism.

Methods: Two biological risk factors for alcoholism were studied: childhood sleep disturbance and a positive family history of alcohol use disorder. We assessed subjective sleep reports and actigraphy in children with ($N=54$) and without ($N=19$) a parental history of an alcohol use disorder (COAs and NCOAs, respectively) between 7.2 and 12.9 years of age.

Results: COAs reported shorter total sleep time, lower sleep efficiency, more daytime sleep, and a trend towards longer sleep onset latency compared with NCOAs. Subjective and objective problems with sleep continuity were more strongly associated with behavioral problems in the COAs, but not in the NCOAs.

Discussion: Assessment and treatment of sleep disturbances in children, particularly high-risk children, may help to prevent negative behavioral outcomes later in life.

Support: Grants to RA Zucker from the National Institute on Alcohol Abuse and Alcoholism R37 AA07065 and DA Conroy from Michigan Institute for Clinical and Health Research UL1 RR024986.

Disclosure: D. Conroy: None. I. Hairston: None. M. Heitzeg: None. R. Armitage: None. R. Hoffmann: None. K. Brower: None. R. Zucker: None.

66. Human Recreational MDMA/Ecstasy Use is Associated with Altered Amygdala and Hippocampus Activation During Novelty Detection

Ronald Cowan*, Peter Mortensen, Margaret Benningfield, Jennifer Blackford

Vanderbilt University School of Medicine, Nashville, TN

Background: Recreational use of MDMA/Ecstasy, an established serotonergic neurotoxin, continues unabated in Western countries. Recent findings suggest that MDMA may have utility as a psychotherapeutic adjunct in refractory posttraumatic stress disorder, yet recreational MDMA use has been associated with increased anxiety and depression. The amygdala and hippocampus are critically involved in the genesis of anxiety and both receive an extensive serotonergic innervation. Some types of anxiety are related to the brain's reaction to novel situations or stimuli. We therefore used the functional magnetic resonance imaging (fMRI) blood oxygenation level dependent (BOLD) method in conjunction with a novelty detection paradigm to examine amygdala/hippocampal neurophysiology in MDMA users and controls.

Methods: We enrolled 16 abstinent MDMA users (6 Females; 21.88 ± 2.00 years old) and 16 control subjects (10 Females; 22.25 ± 4.39 years old) with no history of DSM-IV mood or anxiety disorders. We used a block design to test amygdala and hippocampal BOLD responses to three types of images: Familiar, Novel Common, and Novel Uncommon. The Familiar images were images that are often seen in real life. Novel Common images represented contextual novelty because the images are common objects (e.g., chair, book) but are novel in the context of the scanner. The Novel Uncommon images represented categorical novelty because the images were unlikely to have been seen before in any context (e.g., fractals). All images had a neutral valence, low arousal ratings, and no human faces. The fMRI paradigm consisted of a familiarization phase in which subjects were shown each of the six Familiar images 16 times. The test phase followed and consisted of two runs in which the order of blocks was counterbalanced across both runs. A 3-T scanner was used to collect EPI images using a sequence optimized for the amygdala and hippocampus. SPM5 and Matlab were used to preprocess the fMRI data, and SPM5 was used to model the experimental design and create contrasts for Novel Common > Familiar, Novel Uncommon > Familiar and Novel Uncommon > Novel Common. Analyses were restricted to the amygdala and hippocampus regions using aal templates. Cluster-based thresholding was used to control for Type I error due to multiple comparisons. Based on simulations calculated with AlphaSim, an uncorrected voxel p-value of 0.05 and cluster sizes of 46 (amygdala) and 94 (hippocampus), provided a corrected $p = 0.05$ for each region. Marsbar was used to extract the stimulus-evoked change in signal intensity for each significant cluster. A t-test of means was used to calculate differences in signal intensity between MDMA users and Controls. To test MDMA dose response effects, a linear regression analysis was performed with MDMA lifetime use (milligrams) as the regressor and amygdala and hippocampus BOLD signal intensity change as the outcome.

Results: MDMA users versus Controls: MDMA users had a larger increase in BOLD signal for the Novel Common images relative to the Familiar images in the both the amygdala (left: $p = 0.02$; right: $p = 0.01$) and the hippocampus (left: $p = 0.01$, right: $p = 0.02$). For the Novel Uncommon versus Familiar contrast, the left hippocampus

exhibited significantly greater increase in signal intensity for MDMA users versus Controls ($p = 0.02$). For the Novel Uncommon versus Novel Common contrast, the right amygdala exhibited a significant difference in signal intensity with Controls > MDMA users ($p = 0.02$), as did the left and right hippocampus (L: $p = 0.02$; R: $p = 0.03$); in this case, MDMA users actually exhibited a decrease in signal intensity for the Novel Uncommon versus Novel Common contrast, while the opposite result was found for the Controls. The left hippocampus also exhibited greater signal intensity increase relative to baseline for Controls than MDMA users in the Familiar versus baseline contrast ($p = 0.02$). Dose-Dependent Effects: Lifetime MDMA use was positively correlated with BOLD signal intensity for both the Novel Common vs. Familiar contrast ($r = 0.52$, $p = 0.04$) and the Novel Uncommon vs. Familiar contrast ($r = 0.58$, $p = 0.02$) in the right amygdala.

Discussion: These initial findings suggest that amygdala and hippocampal neurophysiology are altered in MDMA users and that a greater lifetime exposure to MDMA is associated with greater activation when viewing novel stimuli. These findings, in a cohort of MDMA users without anxiety or depressive disorders, suggest that the fMRI BOLD method may be sensitive to MDMA neurotoxicity that is subthreshold for producing detectable changes in mood or anxiety.

Disclosure: R. Cowan: Part 1; Novo Nordisk, Shire Pharmaceuticals, SWMF Life Science Fund. Part 2; Novo Nordisk, Shire Pharmaceuticals. Part 3; Novo Nordisk, Shire Pharmaceuticals. Part 4; Novo Nordisk, Shire Pharmaceuticals. P. Mortensen: None. M. Benningfield: None. J. Blackford: None.

67. Signatures of Schizophrenia and Vulnerability for Schizophrenia: Disordered Functional Interactions Between Frontal Cortex, the Hippocampus, Striatum and the Amygdala Revealed by fMRI and Dynamic Causal Modeling

Vaibhav Diwadkar*, Mihály Bánya, Neil Bakshi, Patrick Pruitt, Sunali Wadehra, Matcheri Keshavan, Péter Érdi, Simon Eickhoff

Wayne State University SOM, Detroit, MI

Background: Schizophrenia is thought to be characterized by disordered functional interactions between critical sub-circuits in the brain, particularly involving the frontal lobe (Lewis, 1997). The development of these circuits is optimally shaped through healthy development (Uhlhass et al., 2010). Neurodevelopmental hypotheses of schizophrenia (Rapoport et al., 2005) imply that a complex combination of genetic and environmental factors can combine to derail functional interactions in patients (SCZ) and individuals vulnerable to schizophrenia including adolescent offspring (SCZ_{Offspring}) (Diwadkar et al., 2004). Network analyses techniques such as Dynamic Causal Modeling (DCM) permit the direct assessment of functional interactions between brain regions (Stephan, 2010). DCM involves the evaluation of competing network model architectures embodying anatomical and functional priors. Following model estimation, Bayesian Model Selection is used to identify models that best capture functional interactions between task-relevant brain regions. Model parameter estimates (i.e., coupling between network constituents) can be compared to investigate differences between clinical, vulnerable and control groups. Here, using DCM and fMRI, we demonstrate disordered a) fronto-hippocampal coupling in SCZ (during associative learning), b) fronto-striatal coupling (during sustained attention) and c) fronto-limbic coupling (during affective processing) in SCZ_{Offspring}.

Methods: Data were obtained from sixty six subjects (SCZ = 11, SCZ_{Offspring} = 19, Controls = 36; Age Range: 8-34 yrs) using a full body Bruker Medspec 4T. EPI images were collected using an 8-channel head coil (TR = 2/3 s; TE = 30 ms; matrix = 64 x 64; FOV = 240 mm; voxel = 3.8x3.8x4 mm). During associative learning (fronto-hippocampal coupling), SCZ and Controls ($n = 22$) learned associations between objects and locations (Diwadkar et al., 2008) over time. During sustained attention (fronto-striatal coupling), SCZ_{Offspring} and Controls ($n = 44$) tracked the rapid (50 ms) presentation of numbers to detect

the repetitions in the sequence. During affective processing (fronto-limbic coupling), SCZ_{Offspring} and Controls ($n=43$) detected the consistency in facial affect between consecutively presented faces (3s/face; Barbour et al., in press). For learning, a total of 20 DCMs were evaluated embodying interactions between regions in an object-location learning network (visual, inferior temporal, superior parietal, hippocampus and dorsal prefrontal); for attention, a total of 72 DCMs were evaluated in a visual attention network (visual, superior parietal, dorsal prefrontal, basal ganglia, anterior cingulate); for affective processing, a total of 100 DCMs were evaluated in a face and emotion processing network (visual, fusiform, amygdala, ventral prefrontal, dorsal prefrontal). For each subject and task, time series ($p_{\text{effects of interest}} < .05$) from the specified regions of interest were extracted for DCM modeling. Following the estimation of each model in each subject, Bayesian Model Selection in SPM8 identified the best fitting models across subjects for each task (Learning: 440 DCMs; Attention: 3,168 DCMs; Affective Processing: 4,300 DCMs).

Results: Parameter estimates (in 1/s) for intrinsic coupling from the winning DCMs for fronto-hippocampal (learning), fronto-striatal (attention) and fronto-limbic (affect) were compared using independent samples *t*-tests (Stephan, 2010). Relative to controls, a) in learning, SCZ showed significantly reduced fronto-hippocampal coupling (.59 < .74, $t_{19} = 2.27$, $p < .02$); b) in sustained attention, SCZ_{Offspring} showed significantly reduced fronto-striatal coupling (.24 < .13, $t_{42} = 59.8$, $p < .001$) and c) in affective processing, SCZ_{Offspring} showed significantly reduced fronto-amamygdala coupling (.008 < .40, $t_{41} = 37.9$, $p < .001$).

Discussion: Across tasks, adult schizophrenia patients and adolescent offspring of schizophrenia patients showed significant reductions in functional coupling between the frontal cortex and other key task-relevant regions involved in associative learning, sustained attention and affective processing. These results: a) provide direct support for disconnection hypotheses in schizophrenia (Friston, 1998) and vulnerability to schizophrenia; b) demonstrate that disordered functional interactions may emerge in vulnerable adolescents even in the absence of frank symptoms and c) highlight the significant value of sophisticated techniques of network analyses in uncovering signatures of risk and vulnerability in the schizophrenia spectrum.

Disclosure: V. Diwadkar: None. M. Bánya: None. N. Bakshi: None. P. Pruitt: None. S. Wadehra: None. M. Keshavan: None. P. Érdi: None. S. Eickhoff: None.

68. Lethal Forethought: Discounting of Future Monetary Rewards and Attempted Suicide in Late-Life Depression

Alexandre Dombrovski*, Katalin Szanto, Greg Siegle, Meredith Wallace, Steven Forman, Barbara Sahakian, Charles Reynolds, Luke Clark

University of Pittsburgh, Pittsburgh, PA

Background: Suicide cancels one's personal future and is often considered an impulsive act. Indeed, impulsive individuals are more likely to attempt suicide. Yet, it is not clear whether they do so with less planning or determination. Lethal suicidal acts may involve planning and forethought. To gain insight into decision processes that lead to suicide, recent studies have examined the way suicidal individuals make decisions in other contexts. They find that people who attempt suicide make suboptimal choices when faced with risk and ambiguity (Jollant et al, AJP 2005, Dombrovski et al, AJP 2010, Clark et al. Psychology and Aging, in press). Yet, nothing is known about the way suicidal people make decisions about the future. We hypothesized that those who plan and carry out the most serious suicide attempts would weigh the future more while making financial decisions, compared to those who make impulsive suicide attempts. We tested whether this was true in people with late-life depression, a condition associated with serious and premeditated suicidal acts. We employed a behavioral economic paradigm, discounting of future rewards - a phylogenetically conserved choice mechanism sensitive to lesions in the orbitofrontal and ventrolateral prefrontal cortex and in

the ventral striatum, and to manipulation of dopamine and serotonin systems in non-human mammals. In humans, delay discounting has been hypothesized to depend on a distributed system that includes the ventral striatum, medial prefrontal cortex and posterior cingulate cortex {Kable & Glimcher, Nat Neurosc 2007} or, alternatively, on two systems, with paralimbic cortical areas responding to immediate rewards and lateral prefrontal cortex and posterior parietal cortex integrating value across time {McClure et al, Science, 2004}.

Methods: Four groups of depressed participants aged 60 and older were asked to choose between smaller immediate and larger delayed rewards: 15 who made life-threatening suicide attempts (resulting in coma, need for resuscitation, unstable vital signs, penetrating wounds of abdomen or chest, third-degree burns, major bleeding), 14 who made non-life-threatening suicide attempts, 12 who seriously contemplated suicide, and 42 people with depression but no history of suicidal thoughts. Thirty-one psychiatrically healthy controls served as a reference group. For suicide attempts, we assessed the degree of planning, including isolation, timing, precautions against discovery, not seeking help, final arrangements, preparation, and suicide note.

Results: Results suggested that older depressed adults who are willing to delay future monetary rewards commit more serious and premeditated suicidal acts compared to their more impulsive counterparts. An exaggerated preference for immediate rather than larger delayed rewards was observed only in those who had either contemplated suicide or made non-life-threatening attempts ($F[4,113] = 5.9$, $p < .001$, effect size: partial eta squared = 0.18) compared to the two control groups. Exaggerated preference for immediate rewards was also associated with poorly planned suicide attempts ($r = .60$, $n = 29$, $p = 0.001$) and unrelated to hopelessness or future time perspective. This effect was unchanged after accounting for education, global cognitive function, use of psychoactive substances, exposure to psychotropic medications, and possible brain injury from suicide attempts.

Discussion: Our findings suggest that, in the context of depression and stresses of old age (loss, disability, pain), at least two different pathways can lead to attempted suicide. In the first scenario, impulsive, present-focused individuals react to stressors with poorly planned suicidal acts that are less likely to be lethal. Future functional imaging studies of neural substrates of this behavior will need to examine pathology in cortical and striatal areas involved in delay discounting. In the second, more ominous, scenario, people who carefully consider their future carry out well-planned and potentially lethal suicidal acts. Neurocognitive substrates of suicidal behavior unique to this group remain unclear, and may include cognitive inflexibility. Both groups likely share previously described abnormalities in reward-based learning.

Disclosure: A. Dombrovski: None. K. Szanto: None. G. Siegle: None. M. Wallace: None. S. Forman: None. B. Sahakian: None. C. Reynolds: None. L. Clark: None.

69. Probabilistic Reinforcement Learning Deficits in Schizophrenia: Relationship to Amotivation

Erin Dowd*, Deanna Barch

Washington University, St. Louis, MO

Background: Motivational impairments are critical features of schizophrenia that significantly impact functional capacity and are resistant to treatment. A growing body of data suggests that while in-the-moment hedonic experience is intact in schizophrenia (reviewed in Kring & Moran 2008), patients may be impaired in the translation of rewarding experiences into future goal-directed behavior (reviewed in Barch and Dowd 2010). One essential component of this translation is reinforcement learning (RL), a process of particular interest in schizophrenia because it is subserved by the mesolimbic dopamine system, which is thought to be dysregulated in this disease. Several studies have shown deficits in reward-based learning in individuals with schizophrenia (reviewed in Gold et al 2008), as well as altered brain activity during processing of reward information (e.g. Juckel et al

2006). Here, we use fMRI and a probabilistic reinforcement learning task to explore the possibility that an impairment in the ability to use rewarding outcomes to guide subsequent choices may contribute to symptoms of amotivation in schizophrenia.

Methods: 15 individuals with schizophrenia and 15 demographically matched healthy controls underwent two sessions of a probabilistic stimulus selection task (Frank et al 2004). In this task, three stimulus pairs with different probabilistic reinforcement ratios (80:20, 70:30, and 60:40) are presented, and participants must learn by trial-and-error to choose the more frequently reinforced member of each pair. Reinforcement consisted of monetary rewards for correct responses. All participants completed 6 blocks of 60 learning trials, followed by a test phase in which the pairs were recombined and no feedback was given. The two sessions used identical tasks with different stimuli. In addition, a separate sample of 14 patients and 10 controls underwent fMRI during the learning phase of the task. An event-related GLM analysis was employed to examine functional activation during the choice and feedback epochs separately.

Results: Accuracy was examined with repeated measures ANOVA analysis with stimulus pair, block, and session as within-subjects factors and group as a between-subjects factor. This analysis revealed significant main effects of stimulus pair ($p < .001$) and block ($p < .001$), reflecting overall better performance for higher reinforcement ratios and an improvement in performance over time. There was also a significant block X session interaction ($p < .04$), and a trend-level block X session X group interaction ($p < .1$). Post-hoc analyses revealed that patients showed a significant main effect of block within Session 2 ($p < .3$), indicating a significant learning slope over time only in the second session. In contrast, controls showed a significant effect of block in Session 1 ($p < .5$), due to ceiling performance during the second session. On the test phase, patients performed more poorly than controls overall ($p < .001$), with no main effect of session or group X session interaction. In addition, transfer measures of positive RL (frequency of choosing the most highly reinforced stimulus) and negative RL (frequency of avoiding the least reinforced stimulus) were examined. Patients showed impaired performance on both measures ($p < .002$). Neuroimaging results and individual differences analyses examining relationships to symptoms of amotivation will also be presented.

Discussion: Patients did not show significant improvement over time during session 1, but did show such improvement during session 2, suggesting that additional practice with the task may aid in their ability to acquire probabilistic contingencies. However, even with additional practice, patients showed deficits in reinforcement learning performance as compared to controls.

Disclosure: E. Dowd: None. D. Barch: None.

70. Dopamine D2 Receptor C957T Polymorphism Influences Reward-Related Brain Function Relevant to Sensation Seeking in Healthy Adolescents

Erika Forbes*, Neal Ryan, Robert Ferrell, Mary Phillips, Eric Rodriguez, Stephanie Holm, Ronald Dahl

University of Pittsburgh, Pittsburgh, PA

Background: Adolescence is an eventful time in the lifespan, involving continued development of brain function and structure; increased reward-seeking behavior; and changes in affect regulation and executive function. The typical development of neural reward circuits is relevant to a number of adolescent-onset disorders, including affective and substance use disorders. Polymorphisms in dopamine-system genes have been associated with reward-related brain function that is, in turn, linked to reward-related behaviors such as impulsivity (e.g., Forbes et al., 2009). The C957T single nucleotide polymorphism (SNP; rs6277) in the dopamine D2 receptor gene (DRD2) is of particular interest for adolescent reward function because it is associated with reward-related behavior such as adolescent substance use, dysfunctional impulsivity, and incentive learning (Frank & Hutchison, 2009; Colzato et al., 2010; Forbes et al., 2009). This SNP also has a known function in dopamine signaling, with influence on D2 receptor affinity and availability in the

striatum (Hirvonen et al., 2009). Little work has addressed the association of dopamine-system polymorphisms with reward-related brain function or behavior in adolescents, however. The current study examined gene-brain-behavior associations relevant to adolescent reward function by testing the influence of DRD2 C957T genotype on striatal reactivity associated with sensation-seeking.

Methods: Participants were 91 healthy adolescents, age 11-13 (mean age 11.9 years; 51% female), with no lifetime history of psychiatric disorder. Functional magnetic resonance imaging (fMRI) was conducted on a Siemens Allegra 3T scanner during a guessing task with monetary reward. Self-reported sensation-seeking was measured two years after the fMRI assessment using the Sensation-Seeking Scale for Children (Russo, 1991). Preprocessing and analyses were conducted using statistical parametric mapping (SPM) software.

Results: Striatal response to reward was positively correlated with total sensation-seeking score (231 voxels, $t = 3.82$, $p < .001$, Talairach coordinates: -10, -3, 20). Constraining our genotype analyses to clusters in the striatum that were correlated with sensation-seeking, we found that CC homozygotes had greater striatal response to reward outcome than T carriers in a large cluster in the caudate (307 voxels, $t = 4.60$, $p_{FWE} = .001$, coordinates: -10, 10, 11). Genotype groups did not differ for sensation-seeking.

Discussion: These novel gene-brain-behavior results in adolescents indicate that genetic characteristics relevant to dopamine signaling and adolescent substance use have impact on neural response to reward. Furthermore, the neural response examined is directly associated with sensation-seeking, a critical behavioral aspect of reward function that increases with adolescent development. Specifically, adolescents who were CC homozygotes for the C957T polymorphism in DRD2 exhibited greater response than T carriers in the caudate during reward outcome, and the region in which this group difference was observed was also directly associated with sensation-seeking behavior. Because the CC genotype is associated with impulsive reward-seeking under stress (White et al., 2009), smoking reward (Perkins et al., 2008), and alcohol dependence (Krachewski et al., 2009), the results of this study have particular implications for alcohol and substance use disorders, in which initiation typically occurs during adolescence. This pattern of association also highlights the general value of imaging genetics approaches for understanding the altered reward function that is postulated to be a mechanism for the development and pathophysiology of reward-related disorders.

Disclosure: E. Forbes: None. N. Ryan: None. R. Ferrell: None. M. Phillips: None. E. Rodriguez: None. S. Holm: None. R. Dahl: None.

71. Cognition in Older Adults with Bipolar versus Major Depressive Disorders

Ariel Gildengers*, Meryl Butters, Denise Chisholm, Stewart Anderson, Amy Begley, Margo Holm, Joan Rogers, Charles Reynolds, Benoit Mulsant

University of Pittsburgh School of Medicine, Pittsburgh, PA

Background: Bipolar Disorder (BD) and Major Depressive Disorder (MDD) are associated with cognitive dysfunction in older age during acute mood episodes and in remission. Identifying whether there are relevant similarities or differences may help in understanding the neurobiology of the disorders and developing specific interventions for BD versus MDD to prevent, halt, or remediate cognitive dysfunction and functional decline.

Methods: 150 subjects with BD ($n = 30$) or MDD ($n = 120$) ages ≥ 65 years (mean [SD] 74.5 [6.2]) were assessed when euthymic using comprehensive measures of cognitive function and cognitive-instrumental activities of daily living (C-IADL). Cognitive performance of subjects with BD or MDD were compared across four domains and globally. The test results were standardized for comparison using a group of mentally healthy comparators ("controls"; $n = 92$) equated on age and education.

Results: Both subjects with BD and MDD were impaired compared to controls. Subjects with BD were more impaired across cognitive

domains than subjects with MDD. Older age, lower education, greater vascular burden, and BD diagnosis were related to worse cognitive function. Subjects with BD and MDD performed close to the ceiling and did not differ significantly on the C-IADL assessment.

Discussion: In BD and MDD subjects, impairments emerge in performance of the tasks used to assess cognitive function, but not in the well-practiced everyday tasks included in C-IADL assessment. The cognitive profiles of older patients with BD or MDD are similar. However, BD is associated with worse overall performance across all cognitive domains.

Disclosure: A. Gildengers: Part 4; GlaxoSmithKline. M. Butters: Part 1; Northstar Neuroscience, Medtronic, Fox Learning Systems. D. Chisholm: None. S. Anderson: None. A. Begley: None. M. Holm: None. J. Rogers: None. C. Reynolds: Part 4; Forest, Eli Lilly, Pfizer, Bristol-Myers Squibb. B. Mulsant: Bristol-Myers Squibb, Wyeth, Eli Lilly, Pfizer.

72. Utility of Combinations of Biomarkers, Cognitive Markers, and Risk Factors to Predict Conversion from Mild Cognitive Impairment to Alzheimer's Disease and Magnitude of Functional Decline in ADNI Subjects

Terry Goldberg*

Feinstein Institute/AECOM, Glen Oaks, NY

Background: We sought to determine the utility of various classes of markers (e.g., cognition, functional activities), biomarkers (e.g., brain volume measures, CSF measures of tau and Abeta1-42), and individual risk factors (e.g., APOE genotype) in predicting conversion from Mild Cognitive Impairment (MCI) to Alzheimer's disease (AD) in the ADNI database. To the best of our knowledge this is the first study to comprehensively examine the full range of such predictors.

Methods: We analyzed the ADNI database to study three groups: Healthy Controls (HC, N = 197), MCI patients who converted to AD (N = 116), and MCI patients who did not convert (N = 204). We determined the predictive utility of 25 variables from all classes of markers, biomarkers, and risk factors in predicting MCI to AD conversion over a two year period in a series of logistic regression models and effect size analyses. Primary outcome measures were odds ratios, pseudo-R²s, and effect sizes.

Results: The following baseline variables predicted conversion within a two year period: everyday functional activities questionnaire (FAQ inventory), delayed verbal episodic memory, and left middle temporal lobe cortical thickness. Cox regression survival analyses were consistent with these results. Next, in an effect size analysis, we observed that change scores for biomarkers (e.g., CSF Abeta and tau, regional brain volumes) were relatively modest over a two year period of the study, but a change in FAQ was large (ES = .84). FAQ decline and trailmaking b decline accounted for nearly 50% of the predictive variance in conversion.

Discussion: Cognitive markers at baseline, especially those related to memory were robust predictors of one year and two year conversion. Moreover, strikingly and unexpectedly, conversion appeared to be driven less by change scores in neurobiological variables than by a sharp decline in functional ability. This suggested that "caseness" was triggered by functional decline, and not by large shifts in disease neurobiology (which may occur earlier). This set of findings has implications for interpreting neurodegeneration in the MCI-AD transitional phase.

Disclosure: T. Goldberg: Part 1; GSK, Merck.

73. Effects of Cognitive and Neural Negative Biases Present in Dysphoric Volunteers on an Emotional Test Battery and the Associated Modulation of fMRI BOLD Signals

Guy Goodwin*, Kevin Craig, Gerard R. Dawson, Colin Dourish, Poornima Kumar, Elisa Favaron, Catherine Harmer

University of Oxford, Oxford, United Kingdom

Background: Depression is reported to be strongly associated with negative biases in processing emotional information. The current

study aimed to evaluate whether a group of medication-free participants, meeting criteria for sub-threshold depression or major depression (dysphoric group) differed from a non-dysphoric group in neural processing of emotional information using an emotional test battery.

Methods: Males or females aged 18 to 45 years with BDI score of 0-5 or ≥10 at both screening and assessment visits and HAM-D score of <24 at both, were studied using a battery of emotional task sensitive to the affect of words or faces with behavioural or fMRI (BOLD at 3T) measures as outcomes.

Results: Dysphoric participants had increased amygdala activation to fearful faces and threatening words. In addition dysphorics had increased activation in the fusiform area to fearful faces. A reciprocal pattern of activation was observed between dorsal and ventral prefrontal cortex in non-dysphorics and this pattern of activity was reversed in dysphoric participants. Dysphorics were also impaired in recalling positive words from a word categorisation task when compared with controls.

Discussion: Dysphoric participants have negative biases in the neural processing of emotional information that are similar to those reported in studies of depressed patients (Harmer et al 2009). Recruitment of dysphoric participants was fast and efficient compared to recruiting drug free depressed patients from primary or secondary healthcare centres. The results indicate that this dysphoric group can be differentiated from non-dysphoric participants in terms of emotional processing and behaviour. Harmer, C.J., O'Sullivan, U., Favaron, E., Massey-Chase, R., Ayers, R., Goodwin, G.M., Cowen, P., 2009. Effect of Acute Antidepressant Administration on Negative Affective Bias in Depressed Patients. American Journal of Psychiatry, 166, 1178-1184. "This study was supported by the P1 Vital CNS Experimental Medicine Consortium (members AstraZeneca, GlaxoSmithKline, Lundbeck, Organon (a subsidiary of Merck) and Pfizer)".

Disclosure: G. Goodwin: Part 1; AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Cephalon, Eisai, Eli Lilly, GlaxoSmithKline, Johnson & Johnson, Lundbeck, Organon, Pfizer, P1 Vital, Roche, Sanofi-Aventis, Servier, Schering Plough, Wyeth. Part 2; Eli Lilly, Servier. Part 3; Eli Lilly, Servier. Part 4; Sanofi-Aventis, Servier, P1 Vital CNS Experimental Medicine Consortium, Johnson & Johnson, Roche. K. Craig: Part 2; P1 Vital. Part 4; P1 Vital CNS Experimental Medicine Consortium, Johnson & Johnson, Roche. Part 5; P1 Vital. G. Dawson: Part 2; P1 Vital. Part 4; P1 Vital CNS Experimental Medicine Consortium, Johnson & Johnson, Roche. Part 5; P1 Vital. C. Dourish: Part 1; Vernalis. Part 2; Vernalis. Part 4; GlaxoSmithKline, Johnson & Johnson, Servier. Part 5; P1 Vital. P. Kumar: None. E. Favaron: Part 3; P1 Vital. Part 4; P1 Vital CNS Experimental Medicine Consortium, Johnson & Johnson, Roche. C. Harmer: Part 1; AstraZeneca, GlaxoSmithKline, Johnson & Johnson, Lundbeck, Organon, Pfizer, P1 Vital, Roche, Servier, Wyeth. Part 2; P1 Vital. Part 3; P1 Vital. Part 4; P1 Vital CNS Experimental Medicine Consortium, Johnson & Johnson, Roche.

74. The Effects of Child Abuse and Neglect on Cognitive Functioning in Adulthood

Felicia Gould*, Jennifer Clarke, Philip Harvey, Christine Heim, Charles Nemerooff

Miller School of Medicine, University of Miami, Miami, FL

Background: Recent research suggests that early life trauma such as abuse (sexual and/or physical) and neglect produce lasting changes in the CNS that control stress responsivity and emotional regulation (Heim, 2001). Specifically, increased HPA axis and autonomic responses to psychosocial laboratory stress have been found in women with a history of early life abuse and current major depression (Heim et al., 2000). Moreover in laboratory animals and clinical studies, persistent CNS alterations after early life stress have been observed in several neurotransmitter systems, and in structural and functional brain imaging measures. Recently, our group and others have

described genetic polymorphisms that regulate vulnerability to depression and post traumatic stress disorder (PTSD) after exposure to child abuse or neglect. We posited that cognitive deficits, often observed in patients with mood disorders, are due in part to the neurobiological consequences of early life trauma. We explored the hypothesis that the nature and magnitude of cognitive deficits would differ amongst individuals according to the specific type of trauma they experienced.

Methods: The Cambridge Neuropsychological Test Automated Battery (CANTAB) was used to assess neurocognitive functioning in a sample of 93 subjects (60 with a history of early life trauma and 33 without). In the patients with a history of early life stress, 47.3% and 9.3% met criteria for current major depression and PTSD, respectively.

Results: Outcome measures were selected via MANOVA for each trauma subtype and then examined by multiple regression modeling to identify significant associations with trauma history and predictors including current or past history of mood disorder and/or PTSD. Subsequent multiple regression modeling revealed significant associations between trauma status and CANTAB measures.

Discussion: The results indicate that a history of childhood trauma is associated with altered neurocognitive functioning, and that specific patterns may be distinguished according to the specific type of trauma experienced. Additionally, current or past history of mood disorders and/or PTSD were associated with deficient cognitive functioning in some trauma subtypes (i.e., sexual abuse) but not others. Taken together these data suggest that a history of early life trauma results in a cascade of neuronal changes sufficient to produce long-term cognitive deficits that vary according to the type of trauma experienced. Support for this research was provided by MH-58922.

Disclosure: F. Gould: Part 1; Sepracor. Part 4; Sepracor. J. Clarke: None. P. Harvey: Part 1; Astra-Zeneca Pharma, Abbott Labs, Cypress Bio Sciences, Eli Lilly, Merck, Shire, Wyeth Pharma (now Pfizer), Duke University/Neurocog Trials. Part 2; Dainippon Sumitomo Pharma America/Sepracor. Part 4; Pfizer, AstraZeneca. C. Heim: None. C. Nemeroff: Part 1; Astrazeneca Pharmaceuticals, CeNeRx BioPharma, PharmaNeuroBoost, Revaax Pharma, NovaDel Pharma, Inc., Corcept Therapeutics. Part 2; Cenerx, Pharmaneuroboost, Astrazeneca, Novadel.

75. Decision-Making Impulsivity in Young Adult Recreational Gamblers

Jon Grant*

University of Minnesota, Minneapolis, MN

Background: Problem gambling is a significant public health problem characterized by persistent and recurrent maladaptive patterns of gambling. Despite reasonable knowledge of the clinical characteristics of problem gambling, little is known of its antecedents. The objective of this study was to evaluate whether cognitive dysfunction exists in young people at risk of later developing pathological gambling.

Methods: Healthy adolescents and young adults (18-29 years, n = 186) who had gambled five or more times in the past 12 months were recruited via advertisements. Recruits undertook clinical assessment including the Structured Clinical Interview for Pathological Gambling (SCI-PG), and neurocognitive assessment with selected paradigms from the Cambridge Neuropsychological Test Automated Battery (CANTAB). Subjects were grouped according to being at no/negligible risk of subsequent pathological gambling (SCI-PG 0, n = 112) or being at elevated risk (SCI-PG 1-2, n = 73).

Results: Subjects at elevated risk of developing pathological gambling exhibited decision-making deficits, gambling a significantly greater proportion of points ($p = 0.010$) and demonstrating less adjustment of behavior as a function of risk ($p = 0.005$) compared to the control group (Cambridge Gambling Task). Response inhibition, sustained attention, set-shifting, and spatial working memory were intact.

Discussion: These data indicate that selective decision-making impairments, objectively assessed using a test dependent upon orbito-frontal integrity, occur in people at risk of pathological gambling. The results

have potential implications for early detection, and highlight the dissociable nature of different aspects of impulsivity (decision-making versus response inhibition). Future work will assess the predictive value of these markers in longitudinal studies.

Disclosure: J. Grant: Part 4; Forest Pharmaceuticals.

76. DNA Methylation Analysis of Putative Schizophrenia Susceptibility Genes in the Human Dorsolateral Prefrontal Cortex Across the Lifespan

Xavier Guitart*, Shusuke Numata, Tianzhang Ye, Thomas Hyde, Daniel Weinberger, Barbara Lipska, Joel Kleinman

GCAP, IRP, NIMH/NIH, Bethesda, MD

Background: DNA methylation of the cytosine base in CpG dinucleotides is a major epigenetic modification playing an important role in the regulation of gene expression. DNA methylation may play a role in neuropsychiatric disorders such as schizophrenia. The aim of the present study was to study putative schizophrenia susceptibility genes across the lifespan in normal non-psychiatric subjects.

Methods: We studied the DNA methylation level of selected CpG sites in the promoter region of a number of putative schizophrenia susceptibility genes ($n = 74$) in the human dorsolateral prefrontal cortex (DLPFC) of non psychiatric subjects across the lifespan, using a customized GoldenGate methylation assay for Vera Code from Illumina. In addition to that, several genes previously shown to display changes in DNA methylation along the lifespan as well as four CpG sites in chromosome X known to be hypermethylated in women, were used for validation purposes. A total of 108 subjects were studied, including 31 fetal specimens. Batch effects were removed using COMBAT, model selection was performed using a STEP-wise procedure, and statistical analysis was performed using multiple linear regressions on the best fit model. The effect was considered significant if the p-value fell below the $p < 0.008$ threshold, after correcting for multiple testing via the False Discovery Rate threshold (FDR) set at $p < 0.05$.

Results: The studied sites displayed a bimodal distribution with almost 70% having low methylation level across the lifespan (beta values < 0.2) and only 7% of loci were virtually completely methylated across the lifespan (beta values > 0.8). The remaining genes (24%) displayed intermediate methylation status sometimes varying as a function of age. We also observed significant sex differences in methylation of the four CpG sites in the X chromosome ($p < E^{-20}$), all four showing more methylation in females than in males, consistent with X inactivation. Age related differences were also observed in the methylation state for several CpG sites corresponding to 7 to 10 genes out of the 74 potential schizophrenia susceptibility genes. There were 7 genes showing significant changes in methylation over six weeks of the 2nd trimester of gestation (DLG4, CAMK2A, NTRK3, NDEL1, CIT, CNR1, GPR85), 2 genes (CAMK2A, FGF20) showed changes in the methylation status during childhood (0 to 10 years of age), and 10 genes (BDNF, CHRNA4, NDEL1, MOBP, PDE4B, DRD3, KCNH2, FGF20, RELN, PRODH) showed methylation level changes during adult life (more than 11 years of age).

Discussion: In summary, our results show a lifespan change in the methylation state of a number of schizophrenia susceptibility genes, in the DLPFC of non-psychiatric controls. The changes that we have observed suggest the possibility that developmental changes in methylation status also could be related to the pathophysiology of schizophrenia.

Disclosure: X. Guitart: None. S. Numata: None. T. Ye: None. T. Hyde: None. D. Weinberger: None. B. Lipska: None. J. Kleinman: None.

77. Allelic Association of the GluR5 Kainate Receptor Subunit Gene *GRIK1* with Adverse Effects of Topiramate

Albert J. Arias*, Richard Feinn, Henry R. Kranzler, Cheryl Oncken

University of Connecticut School of Medicine, Farmington, CT

Background: Topiramate is efficacious in the treatment of alcohol dependence, with evidence that it may also be useful in the treatment

of nicotine and cocaine dependence, but adverse effects substantially limit its use. Based on an allelic association of *GRIK1* with alcohol dependence (Kranzler et al. 2007), Ray et al., (2009) found a (nominally) significant association between a single nucleotide polymorphism (SNP) rs28322407 in *GRIK1* and severity of adverse effects in a study of non-treatment seeking alcoholics. We sought to replicate that finding in a sample of individuals treated with topiramate for smoking cessation.

Methods: Pharmacogenetic analysis was performed on N=57 subjects participating a 10-week smoking cessation trial. Subjects were randomized to one of three treatment groups: placebo, topiramate, or topiramate plus nicotine patch. The dosage of topiramate was titrated over 4 weeks to a maximum of 200 mg/day, and the topiramate plus patch group received a standard nicotine patch taper starting with 21 mg/day on the quit date (after 2 weeks on topiramate). Adverse effects were assessed at each visit via a standard self-report questionnaire. Subjects were genotyped for rs28322407 and rs2186305, which was also previously associated to alcohol dependence (Kranzler et al. 2007).

Results: The topiramate/nicotine patch group reported significantly more adverse effects than placebo ($F = 5.27$, $p = .006$). However, there was no evidence of association to adverse effects. Rs28322407 showed no main effect of genotype ($F = .14$, $p = .240$) and no interaction between topiramate and genotype ($(F = .012$, $df = 1$, $p = .90$). Rs186305 ($F = 6.64$, $df = 1$, $p = .013$) showed a main effect, but no evidence of a genotype by topiramate interaction ($F = .46$, $df = 1$, $p = .50$).

Discussion: We found no significant association to the severity of adverse effects for the *GRIK1* SNPs examined in this trial of topiramate for smoking cessation. Pharmacogenetic analysis of topiramate adverse effects in larger samples is needed to evaluate the validity of the prior report by Ray et al. (2009) since such effects could help to optimize and personalize topiramate treatment for addictive disorders.

Disclosure: A. Arias: None. R. Feinn: None. H. Kranzler: Part 1; Ortho-McNeil, Forest, Alkermes, Sanofi-Aventis, Solvay, elbion, Lundbeck, Alcohol Clinical Trials Initiative (ACTIVE) is supported by Lilly, Janssen, Schering Plough, Lundbeck, Alkermes, GSK, and Abbott. Receives support from ACTIVE., Gilead Sciences. Part 4; Ortho-McNeil, Merck, Bristol-Myers Squibb. C. Oncken: Part 1; Pfizer, Nabi-Biopharmaceuticals. Part 4; Pfizer, Nabi-Biopharmaceuticals.

78. Retinoic Acid and Affective Disorders: The Evidence for an Association

J. Douglas Bremner*, Kirsty Shearer, Peter McCaffery

Emory University School of Medicine, Atlanta, GA

Background: Isotretinoin (13-cis-retinoic acid, or 13-cis-RA) (Accutane), approved by the FDA for the treatment of acne, carries a black box warning related to the risk of depression, suicide, and psychosis. Retinoic acid (RA), the active form of vitamin A, regulates gene expression in the brain, and isotretinoin is its 13-cis isomer. Retinoids represent a group of compounds derived from vitamin A that perform a large variety of functions in many systems, in particular the CNS, and abnormal retinoid levels can have neurological effects. Although infrequent, proper recognition and treatment of psychiatric side effects in acne patients is critical given the risk of death and disability. This paper reviews the evidence for a relationship between isotretinoin, depression and suicidality.

Methods: Data Sources: Evidence examined includes: 1) case reports; 2) temporal association between onset of depression and exposure to the drug; 3) challenge-rechallenge cases; 4) class effect (other compounds in the same class, like vitamin A, having similar neuropsychiatric effects); 5) dose response; and 6) biologically plausible mechanisms. Study Selection: All papers in the literature related to isotretinoin, depression and suicide were reviewed, as well as papers related to class effect, dose response, and biological plausibility. Data Extraction: Information from individual articles in the literature was extracted.

Results: The literature reviewed is consistent with an association between isotretinoin administration, depression and suicide in some individuals.

Discussion: The relationship between isotretinoin and depression may have implications for a greater understanding of the neurobiology of affective disorders.

Disclosure: J. Bremner: None. K. Shearer: None. P. McCaffery: None.

79. Genome-Wide Association Mapping of Susceptibility Genes for Antipsychotic-Induced Tardive-Dyskinesia in Mice

James J. Crowley*, Corey R. Quackenbush, Amanda L. Pratt, Daniel E. Adkins, Edwin Van Den Oord, Molly A. Bogue, Fernando Pardo-Manuel, Howard L. McLeod, Patrick Sullivan

UNC Chapel Hill, Chapel Hill, NC

Background: Tardive dyskinesia (TD) is a serious adverse drug reaction that affects one in four patients treated with typical antipsychotic medications. It is characterized by repetitive, involuntary, purposeless movements primarily of the orofacial region. Unfortunately, it is currently impossible to predict whether or not patients treated with antipsychotics will develop this side effect, so there is great interest in discovering genetic predictors of susceptibility to this side effect.

Methods: We used a genetically diverse panel of laboratory mice, the Mouse Phenome Project strains, to estimate the heritability of antipsychotic-induced TD and begin to map genetic loci associated with this drug reaction. A total of 27 inbred mouse strains (N=5-8 mice per strain) were chronically treated with haloperidol (3 mg/kg/day via subdermal slow-release pellets) and monitored for the development of vacuous chewing movements (VCMs; the mouse analog of TD) and other movement phenotypes derived from open field activity and the inclined screen test.

Results: Haloperidol-induced VCMs and every other EPS-related phenotype showed an across-time drug response heritability >50%. We next performed genome-wide association mapping using two algorithms (EMMA and TreeQA) and identified a number of loci with significant and suggestive association.

Discussion: We conclude that: 1) mouse VCMs are a valid animal model of antipsychotic-induced TD, 2) TD susceptibility is a heritable trait in mice and 3) genetic mapping of TD susceptibility alleles in mice is a promising approach, one that is likely to be empowered by using a more appropriate mapping population such as the Collaborative Cross.

Disclosure: J. Crowley: None. C. Quackenbush: None. A. Pratt: None. D. Adkins: None. E. van den Oord: None. M. Bogue: None. F. Pardo-Manuel: None. H. McLeod: None. P. Sullivan: None.

80. Mediating Relationships Between Atypical Antipsychotic Associated Metabolic Syndrome, Inflammation, and Folic Acid Metabolism

Vicki L. Ellingrod*, Alan Prossin, Tyler B. Grove, Michael J. Bly, Stephan F. Taylor, Melvin McInnis

University of Michigan, Ann Arbor, MI

Background: The risk of metabolic syndrome with atypical anti-psychotic use (AAPs) is known and may be elevated in MTHFR 677T/ COMT 158Val allele carriers. In general inflammation is garnering much attention for its role in cardiovascular disease (CVD) as well as mental illness. In particular cytokines are associated with depressed mood, but whether this inflammatory marker accounts for the comorbidity of metabolic syndrome is unclear. While the relationship between inflammation and metabolic syndrome development has been examined within the general population, this has not been done within the schizophrenia and bipolar population who are at greater risk for cardiovascular disease. The purpose of this study was to examine the relationship between the MTHFR 677C/T and COMT Val158Met variants, metabolic syndrome, and inflammatory markers in schizophrenia and bipolar subjects treated with AAPs.

Methods: 174 subjects were currently included in this cross-sectional analysis. Subjects were screened for the metabolic syndrome using the NCEP ATP-III criteria, and genotyped for the MTHFR 677C/T, 1298A/C and COMT Val158Met variants. Additionally serum folate, vitamin B12, and homocysteine are measured. IL-6 and TNF-alpha concentrations were determined using standard ELISA techniques within the bipolar group.

Results: Overall, 41% meet metabolic syndrome criteria. The subject's mean age (\pm s.d.) was 45 years, 73% are Caucasian, 51% were male, 66% were receiving clozapine, olanzapine, risperidone or quetiapine, and the mean Body Mass Index (BMI) is $31.2 \pm 7.6 \text{ kg/m}^2$. 47% had a diagnosis of bipolar disorder. 60% were current smokers. There are no differences in age, gender, AAP exposure, or BMI between genotype groups. The risk of meeting metabolic syndrome was 2.24 times higher in subjects receiving AAPs. (95% CI: 95% CI: 1.17 - 4.30, $p = 0.012$). The risk was also related to MTHFR/COMT genotype after controlling for folate exposure ($p = 0.0035$) with a significant interaction between these two variables ($p = 0.01$). Homocysteine concentrations were significantly related to the MTHFR/COMT genotype and folate exposure as well ($p < 0.000$). In subjects with bipolar disorder, IL-6 was significantly greater in the group with metabolic syndrome as compared to the group without. IL-6 was also found to be significantly correlated to the number of criteria of metabolic syndrome across all subjects with bipolar disorder. Using a simple mediation model with AAP use as the independent variable, TNF-alpha as the mediating variable, and waist circumference as the dependent variable, we found that TNF-alpha mediated the effect of AAPs on waist circumference (effect size 3.9, $z(3.98) = 2.3$, $p = 0.02$) for the bipolar subjects. We then attempted to understand the mechanism behind the hyperglycemic effect of AAPs by using a mediation model with two serial mediators ($m_1 = \text{tnf-alpha}$, $m_2 = \text{waist circumference}$), AAP usage as the independent variable, and glucose as the dependent variable. In our model, AAPs conferred an increased risk of elevated glucose, doing so indirectly via TNF-alpha and waist circumference.

Discussion: Overall, our results show a relationship between folate exposure, MTHFR and COMT and metabolic syndrome. Subjects with at least one variant allele for the enzymes involved in folate and homocysteine metabolism have greater than a 2 fold risk of developing metabolic syndrome with AAP use. These data add more clues into folate's role in mental illness and may lead to new treatment options as we work to eliminate the metabolic risks associated with AAP treatment. Additionally, this investigation examined inflammation as it relates to hyperglycemia seen in this population and its mediating role between AAP use and waist circumference. Due to the small sample size, these results should be taken cautiously and need to be confirmed as we recruit additional study subjects. Acknowledgements: This project was supported by the NIMH (R01 MH082784-01) and the NIH-NCCRR, GCRC Program (M01-RR-59 and UL1RR024986), the Chemistry Core of the Michigan Diabetes Research and Training Center (NIH5P60 DK 20572), the Washtenaw Community Health Organization (WCHO), the National Alliance for Research in Schizophrenia and Depression (NARSAD) Independent Investigator Award, and the University of Michigan Prechter Bipolar Genes Study.

Disclosure: V. Ellingrod: None. A. Prossin: None. T. Grove: None. M. Bly: None. S. Taylor: None. M. McInnis: None.

81. Long-term Behavioral Sequelae And Neurobiological Effects Of Adolescent Olanzapine Treatment

Douglas Frost*, Monika Vinish, Jeanne Milstein, Jennifer Enos, Ahmed El-Nabawi, Tim Adle, S. Robson, Istvan Merchenthaler, Bryan Kolb

University of Maryland School of Medicine, Baltimore, MD

Background: Antipsychotic drugs (APDs) are used to treat schizophrenia, bipolar- and autism spectrum disorders. APDs are increasingly used in the treatment of children and adolescents. APDs exert their therapeutic effects largely by modulating monoaminergic

neurotransmission. The monoamine neurotransmitters regulate numerous neurodevelopmental processes. In humans, brain development continues through the 2nd postnatal decade. Thus, early life APD exposure can potentially cause long-lasting, functionally significant changes in neural circuitry. The long-term effects of early life APD exposure are rarely studied and could vary according to the age of exposure because the cerebral substrate upon which the drugs act changes during development.

Methods: We treated colony bred rats with the atypical APD olanzapine (OLA, 7.5 mg/kg/day in 1mM acetic acid) or vehicle, administered in the drinking water from postnatal day 28 (P28) through P49. This dosing protocol induces peak- and trough plasma OLA concentrations that produce dopamine D₂ receptor occupancies in the human therapeutic range. Starting during young adulthood (>P90), the rats were tested to assess aspects of affect, cognition, social behavior, sensorimotor integration, motor skill, learning and memory. Additional OLA-treated and control rats were euthanized at ~6-7 mo and brain tissues were studied by *in situ* receptor binding. **Results:** OLA-treated rats had significant deficits of working memory (delayed non-match to sample test), but not of spatial learning (Morris water maze task). OLA-treated rats were more reactive to handling, trended towards elevated basic acoustic startle responses, had significant deficits in prepulse inhibition and appear to be hyper-responsive to novelty. OLA treatment did not alter social approach. OLA-treated rats did not differ significantly from controls in tests of skilled reaching, anxiety (elevated plus maze), baseline plasma corticosterone or corticosterone response to transient restraint stress. Adolescent OLA treatment also appears to heighten rats' responses to novelty. Additional behavioral measures are under study. In OLA-treated rats, dopaminergic (DAergic) D₁ receptor binding was significantly decreased in the orbital- and medial prefrontal cortices and the nucleus accumbens. The effects of adolescent OLA treatment on DA secretion in the nucleus accumbens are under study. The effects D₂ receptor binding was significantly increased in the medial prefrontal cortex and nucleus accumbens. Total dendritic arbor is decreased in dentate granule cells, but not in CA3 pyramidal cells, of the hippocampus.

Discussion: Together, the behavioral data suggest that adolescent OLA treatment induces a spectrum of long-term behavioral deficits consistent with altered DAergic function, an interpretation supported by our data on D₁ and D₂ receptor binding.

Disclosure: D. Frost: None. M. Vinish: None. J. Milstein: None. J. Enos: None. A. El-Nabawi: None. T. Adle: None. S. Robson: None. I. Merchenthaler: None. B. Kolb: None.

82. Patterns of Benzodiazepine Use From a Nationwide Study in the Grand-Duchy of Luxembourg (1995-2006): Proposed New Definition of High-Dosage Use and Abuse Jean-Marc Cloos*, Paul Koch, Guy Chouinard

ZithaKlinik, Luxembourg

Background: Long-term intake of benzodiazepine is relatively frequent and is associated with abuse liability and dependence. Prevalence rates of benzodiazepine intake vary amongst studies, due to definitions of benzodiazepine use and abuse, lengths of observation period and samples of subject. Objective: To examine short- and long-term clinical use of benzodiazepines in a large national sample over a several-year period, and to compare the data with results from published studies.

Methods: A twelve-year population-based study was conducted in the country of Luxembourg by examining benzodiazepine prescriptions for all insured subjects in the national health system from 1995 (n of insured subjects = 387,862) to 2006 (n of insured subjects = 449,972). Patterns of benzodiazepine use and characteristics of benzodiazepine users were established.

Results: In Luxembourg, 24 benzodiazepines were prescribed for medical indications at the time of the study. The overall number of Defined Daily Dose (DDD) per 1000 insured subjects per year was 82.9;

the five most prescribed (in DDDs/year) were lormetazepam, lorazepam, alprazolam, bromazepam and loprazolam, the last four being high potency benzodiazepines. Alprazolam was the only benzodiazepine showing a threefold increase in its annual prescribed volume during the study period. In the first ten years of the study period, 68 subjects were identified as highest dosage users, meaning ≥ 100 mg/day of an equivalent dose of diazepam for more than 6 years; in this group ($n=68$), the two most frequently prescribed benzodiazepines were bromazepam ($n=42$) and alprazolam ($n=52$), 40 subjects (58.8%) were 50 years and older, and 45 (66.1%) were women (30 older than 50). To evaluate prescription length patterns, all subjects, identified as having had at least one benzodiazepine prescription ($n=236,263$) in the 12-year study period, were divided into 3 groups: 1) A group of "short term delivery", which included subjects with a benzodiazepine prescribing pattern either once or 3 months and less; 34.9% ($n=82,379$) of the 236,263 subjects receiving at least one prescription were classified in this category. 2) A group of "discontinuous delivery", which included subjects who had several prescriptions with at least a one-year break between; 38.4% ($n=90,773$) of the 236,263 subjects were classified in this category. We estimated the total annual dose prescribed for these subjects in the years they were taking benzodiazepines on a yearly average dose \leq than 180 DDDs, equivalent to 2 DDDs/day over a 3-month period, as not problematic; 84,839 (35.9%) of the 236,263 subjects who received at least one benzodiazepine, met this definition; other subjects in this category ($n=5,934$, 2.5% of benzodiazepine subjects) had an average benzodiazepine use higher than 180 DDDs/year during the time they consumed. 3) A group of "continuous delivery", which included subjects who never stopped taking benzodiazepines once prescribed; 63,111 subjects, 26.7% of all subjects with at least one dose, satisfied this category.

Discussion: Our results are comparable to those reported in the literature. However, differences exist in the types of benzodiazepines prescribed and the concept of high-dose abuse. First, we used a cut-off point of 2 DDDs/day to define high-dose use, but thought that there should be a better definition, meaning the use above the benzodiazepine therapeutic dose range, and are proposing a formula to calculate abuse. The sample was examined using this new formula, and a constant number of high dose users was found throughout the 12 years of the study period.

Conclusion: 16.9% of the health insured population in Luxembourg had at least one benzodiazepine prescription per year, 62.1% are women, and 61.6% are aged 50 years or more. 26.7% of the users had long-term prescriptions (> 3 months), mainly (93.2%) at a low dosage (< 2 DDDs/day). High dose use remains relatively rare and was found in a yearly average rate of 5.3% among all benzodiazepine users, or 0.9% of all insured subjects. Alprazolam was the only benzodiazepine showing a threefold increase in its annual prescribed volume.

Disclosure: *J. Cloos:* Part 1; Jean-Marc Cloos has received speaker honoraria from AstraZeneca and GlaxoSmithKline within the last 5 years. *P. Koch:* None. *G. Chouinard:* Guy Chouinard has received consulting fees/honoraria within the last 5 years from Pfizer, Schering Plough, BioLineRx, and Takeda.

83. Long Term Course of Posttraumatic Stress Disorder, Major Depressive Disorder, and Generalized Anxiety Disorder after the 9/11 Attacks: A Longitudinal Examination

Yuval Neria*, Mark Olfson, Marc Gameroff, Margarita Bravova, Priya Wickramaratne, Myrna Weissman

NYSPI/Columbia University, New York, NY

Background: Most trauma-related mental health research has been limited to cross-sectional assessments of posttraumatic stress disorder (PTSD). While the association between psychological trauma and PTSD is empirically well supported, much less is known about relationships between trauma exposure and other psychiatric disorders, such as major depressive disorder (MDD), and generalized

anxiety disorder (GAD). By using a prospective urban cohort of primary care patients in northern Manhattan ($n=469$), assessed approximately one and four years after the September 11, 2001 (henceforth 9/11) terrorist attacks, we estimate the prevalence and course of 9/11-related PTSD (henceforth PTSD), MDD, GAD, in the entire sample, and among subjects who reported loss in 9/11 attacks, including persistence, remission, and late-onset.

Methods: Baseline assessment was conducted between December 2001 and January 2003 and follow-up assessment was conducted between January 2004 and May 2007. The sample consisted of adult primary care patients attending the faculty and resident group practice in general internal medicine at Columbia University Medical Center in New York City. Eligible patients were between 18 and 70 years of age. Of the 474 subjects who were interviewed at both baseline and follow-up, five were omitted from the analyses because of missing status of PTSD, MDD, or GAD at one or more time point. Assessments included the PTSD Check List-Civilian Version (PCL-C; cutoff score 44) and the Primary Care Evaluation of Mental Disorders (PRIME-MD) Patient Health Questionnaire (PHQ) to assess current symptoms of DSM-IV major depressive disorder (MDD) and generalized anxiety disorder (GAD).

Results: At baseline, approximately one year after the 9/11 attacks, prevalence rates were PTSD (10.1%), GAD (13.2%) and MDD (23.2%). At follow-up, approximately 4 years after the attacks, the rates were PTSD (4.6%), GAD (9.4%) and MDD (19.0%). The decline in PTSD was statistically significant ($p < .001$). Within the subsample who reported knowing someone who was killed in the attacks ($n = 133$), prevalence rates at baseline were PTSD (18.7%), GAD (19.7%) and MDD (28.8%) and follow-up rates were PTSD (7.3%), GAD (11.4%) and MDD (22.0%). Again, only PTSD showed a statistically significant decline in prevalence ($p < .05$). Most of the subjects with PTSD (88.6%) and GAD (79.0%), and half of the subjects with MDD (54.0%) had remitted from the disorder at follow-up. Among those with disorders at follow-up, most of the subjects with PTSD (75.0%) and GAD (70.5%), but only a minority with MDD (43.8%) did not have the disorder at the baseline assessment. This pattern was similar within the subsample of subjects who lost someone they knew in the attacks, with PTSD being the least persistent (only 3.3% had PTSD at both time points) and MDD being the most persistent (14.4% had MDD at both time points) of the three disorders.

Discussion: This study examined the long-term trajectories of PTSD, GAD and MDD following the 9/11 attacks in a predominantly low-income, minority, primary care patient cohort. From approximately 1 year after the attacks to approximately 4 years after the attacks, there was a decline in prevalence of all three disorders, although the decline was only significant in PTSD. This pattern is consistent with most previous prospective 9/11 reports showing that the mental health effects of the attacks seem to decline with the passage of time. We also found that, at both time points, MDD was the most prevalent and the most persistent of the three disorders, suggesting that more resources should be devoted to diagnosis and treatment of MDD in the aftermath of disasters. Our data suggest that most patients with baseline PTSD and GAD have remitted before the follow-up assessment and most patients with PTSD and GAD at follow-up had late-onset disorders. Patients with baseline MDD had the lowest rates of remission and the highest rates of persistence compared to PTSD and GAD. These findings suggest that although some mental health problems in the aftermath of disasters such as 9/11 attacks may remit over the long term, late-onset disorders in individuals who were not initially symptomatic may be more common than previously thought. The high prevalence of MDD and GAD in our sample and the fact that neither disorder showed a significant decline in prevalence over time suggest that there should be more focus on these disorders in trauma research. Longitudinal studies with longer follow-up assessments may provide highly needed knowledge with regard to the range of trauma-related disorders beyond PTSD, and their course patterns over time.

Disclosure: *Y. Neria:* Part 4; NARSAD. *M. Olfson:* None. *M. Gameroff:* None. *M. Bravova:* None. *P. Wickramaratne:* None. *M. Weissman:* Eli Lilly & Co.

84. The Father in the Hallway: Differing Parental Roles and Their Relation to Post-Traumatic Stress Reactions in Parents of NICU Babies

William Binder, Lonnie Zeltzer, Charles Simmons, Anand Pandya*

Cedars-Sinai Medical Center, Los Angeles, CA

Background: A recently published extensive literature review identified several important areas of study that are needed in regards to the impact of pediatric critical illness on families. It was found that most family impact data have been obtained exclusively from mothers. We sought to examine the role of premature birth and neonatal intensive care unit hospitalization (NICU) as a causative factor in the development of post-traumatic stress reactions in fathers of NICU babies. It was hypothesized that fathers experience clinically significant levels of post-traumatic stress as has been shown in mothers of NICU babies and that the degree of symptoms experienced is related to the infant's severity of illness.

Methods: 20 father-mother couples of premature (<37 weeks gestation) infants admitted to the NICU were asked to complete a 22-item self report (Impact of Event Scale - Revised) of acute stress symptoms experienced over the previous 7 days. The questionnaire was administered at 4 time points over the post-natal time period. Standardized measures of NICU infant illness severity were applied to assess the correlation between fathers' post-traumatic stress symptoms and the degree of illness in the infant.

Results: A significant number of fathers were found to suffer post-traumatic symptoms after NICU admission. Unexpectedly, we found no correlation between the degree of psychological symptoms experienced by parents and the degree of illness of their infant. However, there was a significant correlation between the degree of psychological symptoms experienced by fathers and those experienced by mothers.

Discussion: Fathers of NICU babies experience similar levels of post-traumatic stress symptoms as do mothers following premature birth. It is difficult to predict which parents will be affected, as parent symptoms do not seem to correlate to the condition of their newborn, either by standardized measures of illness, gestational age, nor birth weight. However, symptoms experienced by fathers were found to correlate highly with those experienced by mothers. These results have important implications for any screening or intervention program aimed at the psychological well-being of parents following premature birth.

Disclosure: W. Binder: None. L. Zeltzer: None. C. Simmons: None. A. Pandya: Part 1; Ortho-McNeil Janssen Scientific Affairs, LLC.

85. Depressive Symptoms Predict Rehospitalization in Schizophrenia

Nina R. Schooler*, Jim Mintz, Peter F Buckley, Donald Goff, Alexander Kopelowicz, John Lauriello, Theo C Manschreck, Alan J. Mendelowitz, Del D. Miller, Daniel W Wilson, Juan Bustillo, Joanne B Severe, John Kane

The Zucker Hillside Hospital, Glen Oaks, NY

Background: The prevalence of persistent depressive symptoms in schizophrenia is well recognized and represents an ongoing treatment challenge. Demoralization, depressed mood and guilt characterize many patients who live in the community. PROACTIVE, an ongoing multi-center RCT comparing a long-acting injectable antipsychotic to oral antipsychotics, provided an opportunity to compare risk of rehospitalization in relation to depressive symptoms in patients with schizophrenia and schizo-affective disorder.

Methods: Brief Psychiatric Rating Scale (BPRS) ratings were available for 295 subjects at baseline; depressive symptoms were defined by ratings on Depressed Mood and Guilt as both a continuous variable (score range 2-14) and categorical (Low 30%, mid 42% and High 30%). At the time of this preliminary analysis a hospitalization event had been recorded for 117 (40%). Proportional

hazard regression models for time to first hospitalization were fitted using depression as a continuous covariate. Other BPRS rated signs and symptoms were entered as a second covariate. Kaplan Meier survival curves for the Low, Mid and High groups were also calculated.

Results: For baseline depression and including the other 16 BPRS items as a second covariate, the effect for depression was significant ($\chi^2 = 3.96$, df = 1, p = .047) and the additional 16 item covariate was non-significant ($\chi^2 = 1.40$, df = 1, p = 0.24). For the three group comparison, the Log-rank ($\chi^2 = 7.63$, p = .022), Wilcoxon ($\chi^2 = 10.45$, p = .005) and likelihood-ratio ($\chi^2 = 8.85$, p = .012) were all significant. We also examined the same depression cluster as a time-varying covariate (i.e., updating the value to the most recent one obtained prior to first hospitalization) and again including the other 16 BPRS items as a second covariate. This model yielded $\chi^2 = 32.3$, df = 1, p < .0001, hazard ratio 1.18. The hazard ratio indicates that the risk of hospitalization increases about 20% with a 1 point increase in the two item depression cluster.

Discussion: These preliminary data come from an eight-site study that is broadly representative of schizophrenia patients in clinical trials. As reported previously (Buckley et al 2009) these patients were in their late 30s, 70% male and 68% Caucasian. They had been diagnosed almost 20 years earlier and had experienced multiple prior hospitalizations. By inclusion criteria they were at high risk for further rehospitalization; within the 12 months prior to study entry they had been rehospitalized or met other criteria indicating need for change of treatment. Thus, the high rate of rehospitalization is not surprising. The importance of our findings is that depressive symptoms both measured at study entry and continuously over the course of the study are an important signal of impending rehospitalization. This suggests that treatment interventions to reduce depressive symptoms will reduce the risk of rehospitalization in this vulnerable patient population.

Disclosure: N. Schooler: Part 1; Abbott Inc, Dainippon Sumitomo, Astra Zeneca, Bristol-Meyers Squibb, Eli Lilly, Hoffman LaRoche, H Lundbeck, Merck, OrthoMcNeil Janssen, Pfizer, Schering Plough. Part 4; OrthoMcNeilJanssen, Astra Zeneca, Bristol-Meyers Squibb, Eli Lilly, H Lundbeck, Pfizer. J. Mintz: None. P. Buckley: None. D. Goff: None. A. Kopelowicz: None. J. Lauriello: None. T. Manschreck: Part 1; OrthoMcNeil Janssen, Pfizer, Inc. Part 4; OrthoMcNeil Janssen, Pfizer, Inc. A. Mendelowitz: None. D. Miller: None. D. Wilson: None. J. Bustillo: None. J. Severe: None. J. Kane: None.

86. The Clinical and Neurocognitive Similarities Between the Schizophrenia and Bipolar Prodromes

Walter Stearns*, Doreen Olvet, Danielle McLaughlin, Andrea Auther, Christoph Correll, Barbara Cornblatt

North Shore-Long Island Jewish Zucker Hillside Hospital, Glen Oaks, NY

Background: There is a strong interest in early intervention strategies for severe mental disorders, with the hope that early intervention can mitigate the impact of the illness itself. Identifying individuals at clinical high-risk (CHR) for illness, as opposed to genetic high-risk (GHR), is one way to identify a group for whom early intervention may be appropriate. There is an increased likelihood that CHR patients may convert to the full-blown disorder compared with GHR for some illnesses. This makes the CHR group a good potential target for early intervention strategies. Individuals at CHR for schizophrenia have been primarily identified by the presence of attenuated positive or negative symptoms i.e. the prodrome, of which approximately 30% convert to schizophrenia. As there has been success in identifying these CHR patients, the understanding of a CHR model for similar illnesses is paramount. A few studies have retrospectively determined that there may be a prodrome to bipolar disorder but this has been scarcely explored in a prospective fashion. As bipolar disorder and schizophrenia may have some overlap in their etiologies and share clinical characteristics, we sought to prospectively determine if there is

a prodrome to bipolar disorder. And if so, could clinical or neurocognitive measures distinguish between the bipolar and schizophrenia prodromes.

Methods: We examined subjects who were initially identified as CHR for schizophrenia and followed them prospectively. Eight subjects developed bipolar disorder, of which five developed psychotic bipolar disorder. Baseline data from subjects who eventually developed bipolar disorder (pre-BP; N = 8), schizophrenia or a psychotic disorder (pre-SZ; N = 24) and a non-converter comparison group (NCC; N = 115) were compared.

Results: The pre-BP and pre-SZ groups did not differ on attenuated positive symptom severity, age of onset, global measures of functioning or on the global neurocognitive score. Compared to NCC individuals, both pre-BP and pre-SZ patients reported more severe attenuated positive symptoms and were more likely to be on antipsychotic medication at baseline. The pre-SZ group had a significantly lower current IQ and was significantly more impaired than the NCC group on the overall neurocognitive score.

Discussion: This study provides preliminary support for a bipolar prodrome, corroborating the retrospective studies to date but ascertained in a prospective manner. When comparing the bipolar prodrome to the schizophrenia prodrome in a CHR for schizophrenia cohort, there are not clear differences between the two prodromes on clinical and neurocognitive measures.

Disclosure: W. Stearns: None. D. Olyet: None. D. McLaughlin: None. A. Auther: None. C. Correll: Part 1; Actelion, AstraZeneca, Bristol-Myers Squibb, Cephalon, Eli Lilly, Ortho-McNeill/Janssen/J&J, GlaxoSmithKline, Hoffmann-La Roche, Lundbeck, Otsuka, Pfizer, Schering-Plough, Supernus, Takeda, Vanda, Boehringer-Ingelheim, Intracellular Therapies, Medicure, Sepracor/Sunovion. Part 2; AstraZeneca, Bristol-Myers Squibb, Cephalon, Ortho-McNeill/Janssen/J&J, Otsuka, Pfizer. Part 3; AstraZeneca, Bristol-Myers Squibb, Cephalon, GlaxoSmithKline, Ortho-McNeill/Janssen/J&J, Otsuka, Pfizer. B. Cornblatt: Part 1; Merck. Part 4; Developed the Continuous Performance Test-Identical Pairs (CPT-IP) version, royalties go to fund Recognition and Prevention Program.

87. Lack of Association Between Paternal Age and Severity of Intermediate Phenotypes in Schizophrenia

Debby Tsuang*, Allen D. Radant, Michael Green, Raquel E. Gur, Laura Lazzeroni, Steve Millard, Larry Seidman, Larry J. Siever, Jeremy Silverman, Neal Swerdlow, Ming Tsuang, David Bruff, Consortium on the Genetics of Endophenotypes and Schizophrenia

University of Washington/VAPSHCS, Seattle, WA

Background: Advanced paternal age has been suggested as a possible risk factor for schizophrenia. Some researchers have hypothesized that *de novo* germline mutations associated with advanced paternal age might account for this association. However, no study has investigated the association between the severity of intermediate phenotypes and advanced paternal age. This analysis investigates whether advanced paternal age is associated with intermediate phenotypes in a large group of schizophrenia research subjects and their unaffected siblings.

Methods: Subjects: As part of the Consortium on the Genetics of Schizophrenia (COGS) protocol, families with at least one person diagnosed with schizophrenia underwent a standardized diagnostic and clinical assessment and a medical record review. Schizophrenia subjects met DSM-IV diagnostic criteria for schizophrenia based on a best-estimate consensus diagnostic procedure that included the Diagnostic Interview for Genetic Studies. Oculomotor tasks: The antisaccade performance of 368 schizophrenia subjects and 485 unaffected siblings was measured using standard oculomotor techniques (e.g., infrared oculographic methods were used to measure subject responses to overlap target and cue stimuli). Subjects performed three blocks of 20 antisaccade trials each, for a total of 60 antisaccade trials. After computerized analysis, at least one oculomotor specialist, who was blinded to participant group, reviewed

all of the tracings. A preliminary variable, which was designated "proportion of interpretable trials," was defined as the total number of antisaccade trials minus the tracings that could not be analyzed due to artifacts, divided by the total number of antisaccade trials. The primary outcome measure, proportion correct or "PPgood," was defined as the number of correct antisaccade responses divided by the number of interpretable saccades. Statistical analysis: Paternal age was defined as the father's age when the participant was born. The proportion of correct saccades was analyzed separately with linear mixed-effects models. The initial model for the proportion of correct antisaccades included paternal age, with and without the potential effects of study site and age. Subjects with schizophrenia and their unaffected siblings were analyzed using separate models.

Results: Valid oculomotor data and paternal age were obtained on 233 schizophrenia subjects and 309 unaffected siblings. The mean + SD of paternal age was 30.2 + 5.5 for the schizophrenia subjects and 30.1 + 5.2 for the unaffected siblings. The linear model indicated no significant relationship between paternal age and PPgood (either by itself or when age and site in the model) in schizophrenia subjects ($p = 0.11$). A separate linear mixed-effect model for unaffected siblings also demonstrated no association between paternal age and PPgood ($p = 0.94$).

Discussion: This study operated under the initial hypothesis that paternal age may serve as a proxy for the likelihood of *de novo* mutations, thereby increasing the risk for developing both schizophrenia and severe intermediate phenotypes. However, no correlation was found between the severity of intermediate phenotypes and paternal age in schizophrenia subjects or in their unaffected siblings. Despite these findings, the association between affected and unaffected siblings for a given intermediate phenotype may differ depending on the age of the father at the time when the affected child was born. Future studies with larger sample sizes are necessary to address this possibility.

Disclosure: D. Tsuang: None. A. Radant: None. M. Green: Part 1; Abbott Laboratories. Part 2; Cypress. Part 3; Dainippon Sumitomo Pharma. Part 4; Lundbeck. Part 5; Otsuka. R. Gur: None. L. Lazzeroni: None. S. Millard: None. L. Seidman: None. L. Siever: None. J. Silverman: None. N. Swerdlow: None. M. Tsuang: None. D. Bruff: None.

88. Social and Cognitive Functioning as Risk Factors for Suicide: A Historical-Prospective Cohort Study

Mark Weiser*, Gad Lubin, Nomi Werbeloff, Eyal Fruchter, Haim Knobler, Michael Davidson

Sheba Medical Center, Ramat Gan, Israel

Background: Previous studies have shown that poor cognitive and social functioning are associated with increased risk of suicide. This study aimed to examine the association between social and cognitive functioning in adolescence and later completed suicide using a historical-prospective design.

Methods: Utilizing a historical prospective cohort design, data from the Israeli Draft Board Register for 756,223 Israeli male adolescents aged 16-17 was linked to data from a death registry, enabling up to 20 year (mean 10.4 year) follow-up for completed suicide (N = 993).

Results: Poor cognitive and social functioning were both associated with increased risk of later suicide (adjusted HR = 1.44, 95% CI: 1.18-1.76 and adjusted HR = 2.3, 95% CI: 1.53-3.4, respectively). A similar trend was observed among adolescents with high IQ (adjusted HR = 1.25, 95% CI: 1.0-1.55). Regardless of their level of social functioning, adolescents who functioned one standard deviation or more below their siblings had increased risk of later suicide (adjusted HR = 1.41, 95% CI: 1.09-1.82).

Discussion: In adolescent males, poor cognitive and social abilities are associated with a slightly increased risk of later suicide. Male adolescents who function poorly compared to their brothers are also at increased risk for later suicide. These data underscore the importance of cognition

and social abilities in understanding the phenomenon of suicide, and particularly indicate the significance of sibling rivalry in the etiology of suicide. However, due to the low prevalence of suicide these characteristics do not enable prediction of later suicide.

Disclosure: M. Weiser: None. G. Lubin: None. N. Werbeloff: None. E. Fruchter: None. H. Knobler: None. M. Davidson: None.

89. Characterization of SERT Function in Mouse Lymphocytes Using Flow Cytometry

Brendan S. Beikmann, Beatriz Campo-Fernandez, Yogesh S. Singh, Stefanie C. Altieri, Ian D. Tomlinson, Sandra J. Rosenthal, Randy Blakely, Anne M. Andrews*

University of California, Los Angeles, Los Angeles, CA

Background: The serotonin transporter (SERT) plays a role in the treatment of multiple neuropsychiatric disorders including depression and anxiety disorders. A 43-base pair insertion/deletion polymorphism known as the serotonin transporter linked polymorphic region (5-HTTLPR), which has been shown to regulate SERT function in transformed human lymphoblasts and rhesus monkey lymphocytes, may also be involved in the efficacy of and treatment response to serotonin reuptake inhibitors (SRIs). Primary human lymphocytes have been shown to express SERT, however studying SERT function in these cells is complicated by the large number of SERT genetic polymorphisms. To reduce this variation, mice with constitutive SERT deficiency were studied. The fluorescent compound IDT307 has been previously shown to be a substrate for SERT, as well as the dopamine transporter (DAT) and the norepinephrine transporter (NET). The aim of the present experiments is twofold. First, we are evaluating lymphocytes as peripheral biomarkers for determining differences in neuronal SERT function by using mice with controlled constitutive SERT deficiency. Second, we plan to determine the applicability of using IDT307 and flow cytometry to measure SERT function in human lymphocytes.

Methods: To determine if flow cytometry can be used to measure SERT function, HEK293 cells transfected with SERT (HEK-SERT) were initially investigated. Furthermore, spleens of wild type and SERT deficient mice were obtained. Lymphocytes (splenocytes) were incubated with IDT307 either immediately after isolation (resting) or after 48 hours of exposure to phorbol 12-myristate 13-acetate (PMA) and calcium ionomycin (activated). Flow cytometry was used to quantify relative fluorescence associated with IDT307 uptake in mouse lymphocytes. Confocal imaging was used to examine visually IDT307 fluorescence in lymphocytes.

Results: HEK-SERT cells displayed time-dependent IDT307 associated fluorescence. This fluorescence was nearly abolished in the presence of the SERT inhibitor paroxetine or when non-transfected HEK293 cells were used. Resting lymphocytes also showed IDT307 associated fluorescence that was sodium and temperature dependent, however no decrease in fluorescence was observed in the presence of SERT, DAT or NET inhibitors. Activation of lymphocytes significantly increased IDT307 associated fluorescence, however it has yet to be determined if this increased uptake is SERT mediated.

Discussion: Flow cytometry and IDT307 can be used to measure SERT function in HEK-SERT cells. IDT307 uptake in resting lymphocytes is sodium and temperature dependent but is not likely mediated by SERT. As compared to resting lymphocytes, activated lymphocytes exhibit greatly increased IDT307 fluorescence. Current experiments are being conducted to determine the role of SERT in activated lymphocyte IDT307-mediated uptake. Determining if lymphocytes are surrogate biomarkers for neuronal SERT function has important implications for future treatment of SERT-related disorders. Overall, our findings suggest that resting mouse lymphocytes are poor models for studying SERT function and they may contain other transporters for which IDT307 is a substrate. Determining whether activation increases SERT function in lymphocytes is an important next step in determining optimal conditions for measuring SERT function in human lymphocytes. Our laboratory is currently working to determine if SERT function is

increased in activated lymphocytes and if so, whether or not activated lymphocyte SERT function correlates with neuronal SERT function.

Disclosure: B. Beikmann: None. B. Campo-Fernandez: None. Y. Singh: None. S. Altieri: None. I. Tomlinson: None. S. Rosenthal: None. R. Blakely: Part 1; Lundbeck, Jubilant Innovation. Part 2; none. Part 3; none. Part 4; Research Contract: Forest Research Institute. Part 5; none. A. Andrews: None.

90. Altered Behavioral Response to Ethanol in NR2B NMDA Receptor Conditional Knockout Mice

Howard C. Becker*, Kimberly A. Badanich, Tamara L. Doremus-Fitzwater, Patrick J. Mulholland, L. Judson Chandler, Eric Delpire

Medical University of South Carolina, Charleston, SC

Background: It is well established that NMDA glutamate receptors play a significant role in mediating a wide variety of pharmacological effects of acute and chronic ethanol. The subunit composition of NMDA receptors is known to influence their pharmacological properties and it has been postulated that the NR2B subunit confers greater sensitivity to the modulatory effects of ethanol. This study examined behavioral responses to acute ethanol challenge as well as voluntary ethanol drinking in a mouse model with genetic deletion of the NR2B subunit. Since genetic deletion of the NR2B NMDA receptor subunit is lethal, conditional NR2B knockout mice were constructed that delay deletion to test the role of this subunit in ethanol-related behaviors.

Methods: Mice lacking the NR2B gene (KO) were produced by mating double-floxed (NR2B[f/f]) mice with CAMKIIa-driven tetracycline transactivator (tTA) transgenic mice and tet-operator (tetO)-driven CRE recombinase transgenic mice. Then, interbreeding these mice produced four unique genotypes: NR2B[f/f]; CAMKIIa-tTA-NR2B[f/f]; tetO-CRE-NR2b[f/f]; and CAMKIIa-tTA-tetO-CRE-NR2b[f/f]. These genotypes are denoted as WT, CAM, CRE, and KO, respectively. Adult male and female offspring representing each of these resultant genotypes ($N = 8-16/\text{sex/genotype}$) were tested for baseline (saline) locomotor activity in an open field, open field locomotor activity following acute low dose (1.5 g/kg) and high dose (3.0 g/kg) ethanol challenge, and sensitivity to hypnotic effects of ethanol (4 g/kg). A separate cohort of animals ($N = 10-12/\text{sex/genotype}$) were tested for voluntary ethanol (15% vs. water) drinking in a limited access (2 hr/day) procedure. Western blot analyses were performed to evaluate extent of genetic deletion of NR2B in tissue punches from dorsolateral striatum, nucleus accumbens, dorsal hippocampus, and prefrontal cortex from an independent group of mice representing each of the genotypes (WT, CAM, CRE, and KO).

Results: A significant reduction in NR2B protein expression was observed in all brain regions examined in NR2B KO mice in comparison with WT, CAM, and CRE control littermates ($N = 4/\text{genotype}$). NR1 expression levels in NR2B KO mice were also significantly reduced, but no significant changes in NR2A subunit expression levels were observed in any of the genotypes with the exception of a minor reduction in the prefrontal cortex of CAM mice. Male and female mice lacking the NR2B subunit exhibited significantly higher levels of basal (saline) locomotor activity in comparison to other genotypes in a novel open field arena. NR2B KO mice also displayed an exaggerated locomotor stimulant response to low dose (1.5 g/kg, ip.) ethanol challenge, while this dose produced a marginal stimulant response in control genotypes. All genotypes showed reduced activity following administration of a high dose of ethanol (3.0 g/kg, ip.). Given the higher baseline (saline) activity in KO mice, this depressant locomotor effect was greater (relative to baseline levels) in NR2B KO mice compared to control littermates. NR2B KO mice were more sensitive than controls to the hypnotic effects of ethanol, as evidenced by longer duration of loss of the righting reflex (LORR) following 4.0 g/kg ethanol. Finally, although females consumed more ethanol (g/kg) due to lower body weights, there was no difference in

ethanol intake across genotypes. Mean \pm s.e.m ethanol intake (g/kg): 1.52 ± 0.13 , 1.51 ± 0.16 , 1.63 ± 0.17 , and 1.52 ± 0.20 for male WT, CAM, CRE, and KO mice, respectively; 2.74 ± 0.17 , 2.64 ± 0.22 , 2.45 ± 0.15 , and 2.51 ± 0.24 for female WT, CAM, CRE, and KO mice, respectively.

Discussion: Results from this study indicate that compared to control littermates, NR2B KO mice exhibit greater baseline (saline) locomotor activity in a novel open field arena, enhanced locomotor stimulant response to low dose (1.5 g/kg) ethanol challenge, and an augmented response to the sedative/hypnotic effects of higher (3.0, 4.0 g/kg) challenge doses of ethanol. However, genetic deletion of the NR2B NMDA receptor subunit does not appear to alter voluntary ethanol drinking behavior under limited access conditions. Collectively, these results suggest that the NR2B NMDA receptor subunit plays a role in mediating/modulating both stimulant and depressant actions of ethanol. Ongoing studies are evaluating ethanol pharmacokinetics in these mice, and ethanol drinking following repeated cycles of chronic intermittent ethanol exposure, as well as characterizing sensitization of ethanol locomotor activity following repeated low dose ethanol administration. Supported by grants U01 AA014095, T32 AA007474, and VA Medical Research.

Disclosure: H. Becker: Part 1; Eli Lilly. K. Badanich: None. T. Doremus-Fitzwater: None. P. Mulholland: None. L. Chandler: None. E. Delpire: None.

91. Cross-Species Genetic Variation and Stress-Induced Epigenetic Regulation of the BDNF Gene: Effects of Stress and BDNF Genotype on Early Infant Development

Stephen G. Lindell*, Qiaoping Yuan, Zhifeng Zhou, Stephen J. Suomi, J. Dee Higley, David Goldman, Christina S. Barr

NIAAA, Rockville, MD

Background: Adaptations to unpredictable or stressful environmental conditions can occur at both the species and the individual levels. Identification of genes that are critical to stress adaptation may be achieved by searching for presence of functionally similar variants that occur in multiple species. Searching for genes at which there is both shared genetic variation and epigenetic regulation may be a particularly powerful approach for identifying genes that play critical roles in stress adaptation, development and survival.

Methods: Brains ($N=16$) were archived from rhesus macaques (*M. mulatta*) that were raised either by their mothers (MR) or in peer- only groups (PR), a model of early adversity. Hippocampal samples were later dissected for DNA and RNA isolation. Chromatin Immunoprecipitation was performed with an antibody against H3K4me3, which marks active promoters. ChIP-Sequencing and whole transcriptome sequencing were performed in parallel on a Illumina GAII sequencer. For select genes, confirmation of newly identified SNPs was performed by dideoxy sequencing. Based on these results, genotyping was performed for a nonsynonymous BDNF SNP. Because of BDNF's role in neuronal plasticity, we wanted to determine whether there were effects of rearing condition and BDNF genotype on results from Brazelton neonatal assessments performed across early development. Effects of rearing condition and genotype on Orientation, State control, Motor Maturity, and Activity Clusters (assessed at P8, P14, P21, and P30) were analyzed using repeated measures ANOVA.

Results: While fewer than 1,000 rhesus SNPs have been annotated, we identified more than 500,000 sequence variants using this method. Non-synonymous SNPs that were present in regions orthologous to those in which non-synonymous SNPs are present in humans were identified (32 in number). We also identified regions in which binding of H3K4me3 was increased as a function of early life stress exposure. Among the genes in which both conserved genetic variation and epigenetic regulation were present was the BDNF gene: there was a non-synonymous SNP in the pro-BDNF domain (Val46Met), and H3K4me3 promoter binding was increased in hippocampus of PR animals. Early stress exposure produced effects in all clusters, and there were interactions between early stress and developmental timepoint for Orientation, State control, and Activity. In each case, PR infants had

higher rates of maturation than did MR infants ($P < 0.001$). There were also both main and moderating effects of Val46Met genotype. The Met allele predicted differences in Orientation ($F(1,80) = 3.8$, $P = 0.05$), Motor Maturity ($F(1,81) = 4.79$, $P = 0.03$), Activity ($F(1,63) = 9.5$, $P = 0.003$) and, at the trend level, State Control ($F(1, 81) = 3$, $P = 0.08$). Effects of allelic variation on Motor Maturity and Activity were moderated by rearing condition ($F(1,63) = 6.9$, $P = 0.01$) $F(1,81) = 3.9$, $P = 0.05$), with allelic effects being more pronounced among PR monkeys. PR monkeys with the Val/Val genotype had higher scores for Motor Maturity and Activity, whereas PR monkeys carrying the Met allele did not differ from MR Val/Val infants.

Discussion: BDNF is a neurotrophin critical for neuronal survival, development and plasticity. We identified a BDNF SNP in rhesus that is similar in location to the human Val66Met SNP, which has been shown to predict individual differences in cognition and susceptibility to psychiatric disorders. We find that there is increased H3K4me3 binding in response to early stress. This could be considered a "predictive adaptive response," as it would translate into increased readiness to express BDNF. We observe accelerated maturation across early development for various neonatal assessment parameters in early stress exposed monkeys and find that effects of rearing condition are moderated by BDNF genotype. It has been proposed that the human loss-of-function Met66 allele has been maintained by balancing selection, and some studies indicate there to be recent positive selection for this and other BDNF variants. It may also be that stress-induced epigenetic regulation of BDNF permits loss-of-function variation to be maintained in selected human populations.

Disclosure: S. Lindell: None. Q. Yuan: None. Z. Zhou: None. S. Suomi: None. J. Higley: None. D. Goldman: None. C. Barr: None.

92. Alcohol Induced Epigenetic Modifications In A Post-dependent Rat Model

Estelle Barbier, Logan Christensen, P Salvatore, Annika Thorsell*, S Kornel, Markus Heilig

NIAAA, NIH, Bethesda, MD

Background: Previous studies have shown that 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, which exert lasting control over gene expression without altering the genetic code, may play a role in several diseases including drug addiction. We hypothesized that epigenetic alterations including DNA methylation and histone modifications may be implicated in neuroadaptations induced by prolonged alcohol exposure. We measured the histone acetyltransferase (HDAC) and DNA methyltransferases (DNMT) activities in several brain regions (hippocampus (hipp), amygdala (amg), prefrontal cortex (PFC), nucleus accumbens (Nac) and paraventricular nucleus (PVN)) from control and "alcohol dependent" rats. Specifically, the expression of glucocorticoid receptor (GR) has been shown to be regulated, at least in part, by DNA methylation and histone acetylation on the GR17 gene promoter. Glucocorticoids control the HPA axis, which plays a role in alcohol consumption, by negative feedback from hippocampus and positive feedback from amygdala. Therefore, we assessed the effect of prolonged alcohol exposure on DNA methylation within the GR gene promoter. In parallel, we also wanted to assess the impact of alcohol exposure on DNA methylation on a larger scale by performing a genome-wide study of DNA methylation.

Methods: To examine the alcohol effects on epigenetic alterations, we exposed rats for 7 weeks in alcohol vapor chambers. DNMT and HDAC activities were measured using a colorimetric quantification through an ELISA-like reaction. The DNA methylation within the GR gene promoter was studied using sodium bisulfite mapping. The DNA methylation sequencing was performed using ABI SOLID technology.

Results: The measurement of both DNMT and HDAC activity shows that neither DNMT nor HDAC activity is altered by alcohol exposure in

Nac, PVN, or PCX. In contrast, the HDAC activity is significantly decreased in the amg. This decrease of HDAC activity (22.6%; $p = 0.006$) may result in an increase of histone acetylation which in turn can increase the expression of specific genes. Furthermore, we observed an increase of both DNMT and HDAC activity in the hippocampus (respectively, 123%; $p = 0.01$ and 19%; $p = 0.002$), which suggest a decrease of gene expression in this brain structure. We found, in amg but not in hippocampus, that repeated cycle of ethanol intoxication and withdrawal decreased the methylation on the GR promoter at a CpG site within the binding sequence for the transcription factor SP1. We observed that SP1 binds this specific sequence only when the CpG site is unmethylated. These data suggest that decreased cytosine methylation at this CpG site could result in an increase in GR expression by increasing the binding of SP1 at the GR gene promoter. However, the mRNA level of GR is not altered after prolonged alcohol exposure, as measured by quantitative nuclease protection assay.

Discussion: The alteration of HDAC and DNMT activity induced in our post-dependent rat model compared to the control rats suggests that epigenetic alterations could mediate some of the neuroadaptations induced by prolonged alcohol exposure. The association of the genome-wide study of DNA methylation with the transcriptome-wide study currently in process will help us to better understand the impact of epigenetic mechanisms on the long-term alterations of genes expression induced by alcohol exposure.

Disclosure: E. Barbier: None. L. Christensen: None. P. Salvatore: None. A. Thorsell: None. S. Kornel: None. M. Heilig: None.

93. Gene Expression Profiling In The Alcohol-Dependent Rat

Jenica Tapocik, Wolfgang Sommer, Melanie Schwandt*, Annika Thorsell, Markus Heilig

National Institutes of Health, Bethesda, MD

Background: Molecular adaptations in the prefrontal cortex (PFC), amygdala (Amyg), and nucleus accumbens (NAc) have been implicated in alcohol dependence, but their exact nature remains largely unknown. The objective of this study was to determine gene expression changes and gene-gene networks in the PFC, Amyg, and NAc of alcohol-dependent rats to further our understanding of the genes changes associated with alcohol dependence.

Methods: Rats were exposed to alcohol vapor for 7 consecutive weeks. BAC levels were taken weekly. Three weeks after exposure, the PFC, Amyg, and NAc were harvested using the atlas of Paxinos and Watson as reference. Tissue from the three brain regions was harvested for gene expression analysis on an Affymetrix Rat Array for vapor exposed (dependent) and non-vapor exposed (non-dependent) animals ($n = 8$ group). Bioinformatics analysis (Ingenuity Pathway Analysis) and the miRNA Sanger Database were used to determine gene-gene interactions, pathways and functions, and networks involved in alcohol dependence.

Results: Gene expression profiling identified 853, 764, and 397 gene changes in the PFC, Amyg, and NAc ($p < 0.05$) associated with alcohol dependence. Alcohol dependence affected a broad range of genes and several biological pathways including cell-cell signaling, neurological disease, axonal guidance signaling, glutamate receptor signaling, nervous system development and function, and cell death. Interestingly, bioinformatics analysis suggested that the microRNA, miR-1, may downregulate 6 genes (BDNF, EML4, SRXN1, ARF-3, HSPA1A, NFKBIE, RNF138) in the PFC and 3 genes (OAT, EML4, HSPA1A) in the Amyg of alcohol dependent animals. Furthermore, miR-124 was found to potentially upregulate 4 genes (GNG10, G3BP1, NFIC, C12ORF23) in the NAc and 2 genes (MTPN, CLDND1) in the Amyg. The Sanger Database for predicted mRNA targets for miRNAs confirmed that miR-1 and miR-124 target the 3' UTRs of these genes.

Discussion: RT-PCR confirmation, miRNA expression profiling, and cell culture studies are currently being carried out to verify the role of miR-1 and miR-124. Our results and current research suggest that some of the long-term effects of alcohol use may be due to their ability to remodel neurons, which presumably reflects a drug-induced reorganization of

synaptic inputs. The gene changes that cause reorganization may be due to the involvement of microRNAs. miR-124 is the most abundant microRNA expressed in neuronal cells and has been previously shown to be involved in neurogenesis. miR-1 has not previously been shown to be involved in brain function, but has been shown to regulate multiple genes involved in synaptic plasticity. MicroRNA regulation of downstream target transcripts may play a pivotal role in long term neuroadaptations underlying alcohol dependence.

Disclosure: J. Tapocik: None. W. Sommer: None. M. Schwandt: None. A. Thorsell: None. M. Heilig: None.

94. The Node of Ranvier in Schizophrenia Postmortem Brain Tissue

Vahram Haroutunian, Panos Roussos*, Pavel Katsel, Larry J. Siever, Kenneth Davis

Mount Sinai School of Medicine, New York NY

Background: Structural, molecular and functional changes in oligodendrocytes have become a major focus of interest in the search for the neurobiological foundations of schizophrenia. There is accumulating evidence of white matter abnormalities and altered expression of myelin/oligodendrocyte-related genes and proteins. Glial signals, including glial soluble factors and paranodal axoglial junctions are required for the formation of the nodes of Ranvier a prerequisite for saltatory nerve conduction. The node of Ranvier is enriched in voltage-gated sodium and potassium channels complexed with neural cell adhesion molecules and axonal cytoskeletal scaffolds. We tested the hypothesis that schizophrenia presents with alterations in the expression levels of oligodendroglial and neurons genes associated with the formation and maintenance of nodes of Ranvier.

Methods: Brain tissue specimens were derived from the Brain Bank of the Department of Psychiatry of the Mount Sinai School of Medicine (New York, NY)/J.J. Peters VA Medical Center (Bronx, NY). For exploratory studies, fifteen cerebral cortical regions (BA: 8, 10, 44, 46, 4, 23/31, 24/32, 20, 21, 22, 36/28, 38, 7, 17 and the hippocampus) from 16 schizophrenic patients and 19 normal comparison subjects were analyzed using independent Affymetrix (Santa Clara, CA) HG-U133AB and HG-U133 Plus2 GeneChips. Hypothesis driven studies used qPCR to measure mRNA levels of nodes of Ranvier genes in the superior temporal gyrus (BA 22) and primary visual cortex (BA 17) from an independent cohort of 22 persons with schizophrenia and 24 unaffected comparison subjects. T-scores and fold changes (FC) were used as a standardized measure of gene expression change for each individual transcript in microarray studies. Two-tailed Student's t-tests were used to compare relative mRNA expression levels in qPCR experiments.

Results: Microarray data (contrast analysis t-scores) from all of the brain regions examined showed an overall dysregulation of axonal and/or oligodendrocytal genes involved in the development, organization and maintenance of nodes of Ranvier. Gene ontology analysis suggested that most of these target genes participate in cell adhesion or ion transport, including genes localized in distinct domains [Axogliaosome, Node, Paranode, Juxtaparanode]. In BA 22 of the independent cohort, the brains of persons with schizophrenia had significantly reduced Ankryin G [ANK3] ($p = 0.01$; FC = -1.24), Neurofascin [NFASC] ($p = 0.016$; FC = -1.33), Neuronal cell adhesion molecule [NRCAM] ($p = 0.01$; FC = -1.3), Navi.6 Sodium channel, alpha subunit [SCN8A] ($p = 0.04$; FC = -1.34) and Contactin 2 [CNTN2 or TAG1] ($p = 0.08$; FC = -1.3). These changes were gene-specific since the mRNA levels of other nodal proteins; Contactin 1 [CNTN1], Contactin associated protein-like 2 [CNTNAP2 or CASPR2], Tenascin-R [TNR], Gliomedin [GLDN] or Navi.2 Sodium channel, alpha subunit [SCN2A] did not show any significant differences among patients with schizophrenia and normal comparison subjects in BA 22 (all $p > 0.19$). Additional specificity was demonstrated by the lack of changes in any of these genes in the primary occipital cortex, BA 17 (all $p > 0.12$). In controls, the mRNA expression levels of ANK3, NFASC, NRCAM and SCN8A were positively correlated with each other, while they were only partially correlated in patients with schizophrenia. The levels of ANK3, NRCAM and Nav 1.6, which are localized to nodal regions of the axolemma

correlated significantly ($rs > 0.4$, $p < 0.04$) with the levels of the neuronal marker ENO2, but not with the expression levels of glial genes.

Discussion: Using a multistep approach we provide strong evidence that in schizophrenia genes responsible for the development, organization and maintenance of nodes of Ranvier are downregulated in the superior temporal gyrus but not primary visual cortex. We found that four nodal genes, namely Ankyrin G, Neurofascin, NrCAM and Nav1.6 sodium channel were downregulated in patients with schizophrenia. The abnormal expression of Ankyrin G appears particularly important since it functions as a membrane protein scaffold and interacts with Neurofascin, NrCAM and Nav1.6 sodium channel on the neuronal membrane. Interestingly, the ANK3 gene was recently associated with schizophrenia in a GWAS in a large European cohort. These data provide mechanistic insights into abnormal oligodendrocyte and myelin-associated interactions with neurons in schizophrenia relevant brain regions and implicate abnormalities in the nodes of Ranvier as a possible substrate for the disconnectivity syndrome in schizophrenia.

Disclosure: V. Haroutunian: None. P. Roussos: None. P. Katsel: None. L. Siever: None. K. Davis: None.

95. Two HPA Axis Genes, CRHBP and FKBP5, Independently and Additively Interact with Childhood Trauma to Increase the Risk for Suicidal Behavior

Alec Roy*, Colin Hodgkinson, David Goldman, Mary-Anne Enoch

VA Medical Center, East Orange, NJ

Background: Childhood trauma is associated with hypothalamic-pituitary-adrenal (HPA) axis dysregulation. Both factors increase risk for suicidal behavior. Corticotropin releasing hormone (CRH) is a key regulator of the HPA axis through the CRHR1 receptor. The actions of CRH are moderated by a high-affinity binding protein (CRHBP). We hypothesized that *CRHBP* and *CRHR1* variation and the interaction with childhood trauma might influence suicidal behavior. Moreover, there might be an additive effect with *FKBP5*, another HPA axis gene previously associated with suicidal behavior in this dataset.

Methods: African Americans: 398 patients with substance dependence (90% men, 120 suicide attempters) and 432 non-substance dependent individuals (40% men, 21 suicide attempters). Cross-sectional study with DSM-IV lifetime diagnoses (SCID). Haplotype-tagging SNPs were genotyped across *CRHBP* (8), *CRHR1* (9) and for completeness, *CRH* (4) and *CRHR2* (11). *FKBP5* genotypes were available. The Childhood Trauma Questionnaire (CTQ) was administered. Of the 830 genotyped participants, a total of 474 (112 suicide attempters) completed the CTQ.

Results: Three distal *CRHBP* SNPs rs7728378, rs10474485, and rs1500 showed a significant interaction with CTQ score to predict suicide attempt. There was an additive effect with *FKBP5*: in the group exposed to high trauma, the prevalence of suicide attempt was 0.49 in carriers of the *FKBP5* rs3800373 major homozygote, 0.40 in carriers of the *CRHBP* rs7728378 major homozygote and 0.58 in carriers of both major homozygotes. There were significant main effects for one *CRHBP* and one *CRHR1* SNP, both unique to African ancestry.

Discussion: *CRHBP* may predispose, independently and additively, to suicidal behavior in individuals who have experienced childhood trauma.

Disclosure: A. Roy: None. C. Hodgkinson: None. D. Goldman: None. M. Enoch: None.

96. The International Consortium On Lithium Genetics (ConLiGen): An Update

Thomas G. Schulze*, Consortium on Lithium Genetics (ConLiGen)

Department of Psychiatry & Behavioral Sciences, The Johns Hopkins University, Baltimore, MD

Background: In 2008, an international group of researchers on bipolar disorder, psychiatric genetics, pharmacogenetics, and lithium therapy met at the NIMH and established the Consortium on Lithium Genetics (www.ConLiGen.org). The creation of the consortium was

co-sponsored by the International Group for The Study of Lithium Treated Patients (www.IGSLI.org). It was the researchers' goal to facilitate high-quality, adequately-powered analyses of lithium treatment response data that would ultimately allow for consistent results. The initial research focus is the identification of genetic determinants of response to lithium treatment in bipolar disorder, in terms of a composite measure considering frequency and intensity of mood episodes.

Methods: Initially comprising 6 groups from the USA, Canada, and Europe, ConLiGen has now grown to a research consortium of 17 member sites from North America (Canada, USA), South America (Brazil, Colombia), Europe (Czech Republic, Germany, Italy, Poland), and Asia (Japan, Taiwan). The current joint sample of bipolar patients characterized for lithium response and available for genetic studies is close to 1,400 individuals. Several other groups are preparing to join ConLiGen and to contribute further samples.

Results: As of August 2010, the complete ConLiGen sample is being genotyped for a genome-wide association study (GWAS) of lithium response, using Illumina chip technology. Genotyping is taking place both at the NIMH (HumanOmni2.5-Quad) and the University of Bonn, Germany (HumanOmni-Quad). Lithium response is rated using an 11-point scale developed by Martin Alda and colleagues (Grof et al. 2002). The 11-point scale measures the extent to which the observed course during long-term treatment may be attributed to lithium administration. The scale's A score is a composite measure of change in frequency, duration, and severity of illness episodes in the course of lithium treatment. It is weighted by factors that influence the degree to which the observed clinical change is considered to be due to lithium (B1-B5 scores in the scale). Across all centers, we studied the inter-rater agreement using case vignettes. Centers were given written instruction on how to use the scale. This first-tier inter-rater agreement analysis yielded a Cohen's kappa coefficient of 0.70 (on the dichotomous phenotype "responder vs. non-responder" based on a predefined cut-off value). A second-tier analysis on new case-vignettes, following a further training in the use of the scale and the development of refined rating guidelines, is currently underway. In addition to the response phenotype, several other phenotypic variables of interest for refined genotype-phenotype analyses are currently being gathered in a ConLiGen database (e.g. age at onset of mania, depression; life-time history of psychosis; family history of bipolar disorder and of lithium response; suicidality; adverse events during the course of lithium response etc.). We will present data on the distribution of response scores in the combined ConLiGen sample as well as analyses on clinical predictors of response in this sample.

Discussion: What started as a small group of dedicated lithium researchers two years ago, has grown into a large genetic research consortium of international scope. ConLiGen has secured funding from the NIMH and the Deutsche Forschungsgemeinschaft (DFG, Bonn, Germany) to perform the largest GWAS of lithium response in bipolar disorder to date. ConLiGen will continue to invite researchers to join its effort of putting lithium at the forefront of pharmacogenetic research in psychiatry. Groups with cohorts of lithium-treated patients and DNA or willingness to collect DNA are welcome to contact ConLiGen for opportunities to join the consortium. In collaboration with both IGSLI centers and large, long-standing multicenter projects such as the NIMH-funded Pharmacogenomics of Mood Stabilizer Response in Bipolar Disorder study (http://www.pharmgkb.org/contributors/pgmResearchGroups/pgbd_profile.jsp), ConLiGen will be actively engaged in supporting and organizing urgently needed prospective studies of lithium response in bipolar disorder and other conditions.

Disclosure: T. Schulze: None.

97. The Serotonin Transporter Promoter Variant (5-HTTLPR), Stress, and Depression Meta-Analysis Revisited: Evidence of Genetic Moderation

Srijan Sen*, Katja Karg, Kerby Shedden, Margit Burmeister

University of Michigan, Ann Arbor, MI

Background: The initial report of an interaction between a serotonin transporter promoter polymorphism(5-HTTLPR) and stress in the

development of depression is perhaps the best-known and most cited finding in psychiatric genetics. Two recent meta-analyses explored the studies seeking to replicate this initial report and concluded that the evidence did not support the presence of the interaction. However, even the larger of the meta-analyses included only 14 of the 56 studies that have explored the relationship between 5-HTTLPR, stress and depression. We sought to perform a meta-analysis including all relevant studies assessing whether 5-HTTLPR moderates the relationship between stress and depression.

Methods: We searched the PubMed database for articles published by November 2009. We subsequently searched the reference sections of identified primary data and review articles and contacted authors to identify additional studies. In order to perform a more inclusive meta-analysis, we used the Liptak-Stouffer Z-score method to combine findings of primary studies at the level of significance tests rather than the level of raw data.

Results: We included 54 studies and found strong evidence that 5-HTTLPR moderates the relationship between stress and depression, with the 5-HTTLPR s allele associated with an increased risk of developing depression under stress ($p = 0.00002$). When restricting our analysis to the studies included in the previous meta-analyses, we found no evidence of association (Munafo studies $p = 0.16$; Risch studies $p = 0.11$). This suggests that the difference in results between meta-analyses was due to the different set of included studies rather than the meta-analytic technique.

Discussion: Contrary to the results of the smaller earlier meta-analyses, we find strong evidence that the studies published to date support the hypothesis that 5-HTTLPR moderates the relationship between stress and depression.

Disclosure: S. Sen: None. K. Karg: None. K. Shedden: None. M. Burmeister: None.

98. Functional Analysis of the Human Dopamine Transporter Genetic Variances Revealed Dominant and Recessive Actions of its Common Alleles and Significant Influence of Ethnic Background on the Genetic Mode

Elena Shumay*, Joanna Fowler, Nora Volkow

Brookhaven National Laboratory, Upton, NY

Background: Dominance, additivity and epistasis are fundamental genetic attributes that define the relative contribution of each allele to a particular trait, because in any diploid organism this contribution can be highly asymmetrical. In human genetics they are needed to establish genetic variance and heritability, to calculate power- and sample size. Inter-genic interactions of the human dopamine transporter gene (SLC6A3) with genes for monoamine transporters (SERT and NET), genes involved in dopamine (DA) metabolism (COMT), and genes encoding DA receptors (DRD2, DRD4) are well-established; in contrast, intra-genic interactions of the SLC6A3 alleles and haplotypes remains unexplored.

Methods: We tested additive and dominance effects of the SLC6A3 alleles based on 3'-UTR and intron8-classifications using brain's DAT density as endophenotype metrics. We used the physiological definitions of genotype and allele actions and aimed to reveal "physiological" dominance rather than the traditional, mathematical-statistical estimates of genetic variance components. Due to the numerous known and unknown confounding factors and the uncertainty of the models we used a heuristic approach, based on a set of assumptions: Both SLC6A3 VNTRs were considered bi-allelic loci; non-allelic (non-) complementation and inter-locus inter-allelic interactions were disregarded under the condition that there is no variation in the environment. Allele's action was inferred from the values of measured allele indexes.

Results: The model's fit analyses (t-test) supported the 9R- dominant model and its reciprocal the 10R- recessive model (3'UTR) in the caudate and putamen. Analysis of the intron8 alleles, suggests a good fit of the 5R- dominant model and, correspondingly in the putamen

and near-statistical significance for the caudate. ANOVA test yielded the same results. Intra-locus allele interactions and additivity was also inferred from instrumental metrics of allelic performances. We found that while the 9R allele was associated with higher DAT availability across the various brain regions, its effect was non-additive (as expected for a dominant allele, akin, homo-allelic non-additivity). The 10R allele displayed its additive (negative) effect on the endophenotype measure which increases with allele dosage (potential homo-allelic additive (negative) action). The 5R allele of the intron8 variance acted as dominant (positive) across brain regions and, distinctly from the 3'UTR alleles, there is an additive (positive) dosage effect. Since the genetic variances are subordinate to the allele frequencies in the population, we asked a question whether the demographics influence the actions of the SLC6A3 alleles. Comparison of allele actions in a context of the ethnically homogeneous background (African-American (AA)- and Caucasian (CE)- populations) revealed that for both VNTR classifications, additive effects were observed only in AA population and not in CE. Heterozygotes (AA) have the endophenotype values higher than the predicted ones; across all brain regions, being most prominent in ventral striatum.

Discussion: This observation is in line with the theoretical prediction that population bottlenecks decrease the value of additivity. Assuming that the higher DAT brain density is beneficial for individual's fitness, the highest DAT density observed in AA heterozygotes suggests heterosis. Detection of heterosis has direct practical implications suggesting that selection of the study participants could not be limited to matching cases and controls by race and age and further, that allele-based analysis of the SLC6A3 VNTR genotypes (i.e., comparison of 10/10 and 9/10 individuals for 3'UTR) needs to be reconsidered.

Disclosure: E. Shumay: None. J. Fowler: None. N. Volkow: None.

99. The Genetics of Cue-Elicited Cocaine Craving: A Preliminary Report

David Smelson*, Lei Yu, Steven Buysek, Gerardo Gonzalez, David Kalman, Doug Ziedonis

UMASS Medical School/VA Bedford/VISN1, Newton, MA

Background: Cocaine craving is an important component in sustaining ongoing cocaine use. Our group and others have been studying individual variabilities in response to the presentation of laboratory cocaine cues designed to elicit craving. Research shows that while some cocaine dependent patients show increases in craving in response to cues (cue-reactive), others display little or no response (non-reactive) to laboratory cues, with cue-reactivity vs. non-reactivity ranging from approximately 35%-50%. To explore the biological basis of these individual differences, we performed a pilot study to examine the genetic basis of cocaine cue-reactivity.

Methods: Cocaine dependent subjects were recruited from a residential substance abuse treatment program. All subjects who signed informed consent and met eligibility criteria for cocaine dependence underwent a baseline assessment, cue-exposure procedure and blood draw. The cue-exposure procedure included exposing individuals to neutral and active cocaine cues and recording both self report craving and changes in physiology. DNA from blood was genotyped for polymorphisms in a number of candidate genes related to addiction.

Results: The sample included 34 cocaine dependent African American males with a mean age of 48 and a mean history of cocaine use of 20 years. Of the candidate genes examined for association with cocaine cue-reactivity, we saw a statistically significant and robust effect for a SNP each in GABRA2 (coding for GABA-A receptor alpha-2 subunit, nominal $p = 0.001$ corrected for multiple models but not for multiple SNPs) and in OPRM1 (coding for mu opioid receptor, nominal $p = 0.025$ corrected for multiple models but not for multiple SNPs). Moreover, the minor allele of the GABRA2 gene SNP was associated with high cocaine cue-reactivity, with 11 out of the 12 cue-reactive subjects containing the allele. The minor allele of the OPRM1 gene SNP

was associated with non-cocaine cue-reactivity, with 14 of the 18 non-reactive subjects containing the allele.

Discussion: Even with our small cohort-size pilot study, our preliminary results showed cocaine cue-reactivity being significantly associated with polymorphisms in components of the GABA and opioid neurotransmission systems. More intriguing is the contrast between the GABRA2 polymorphism and the OPRM1 polymorphism in cue-reactivity and non-reactivity, suggesting a differential role in these systems. GABAergic synapses constitute the major inhibitory neuronal synapses in the CNS, and GABA neurotransmission is vitally important for proper function of the brain. The opioid system, in addition to being known for mediating the effects of opiate drug actions (morphine, heroin), also mediates inhibitory neurotransmission at the synapses in the brain and the spinal cord. Thus, our preliminary results highlighted a hitherto unidentified possibility – inhibitory neuronal circuitry such as GABA and opioids may play an important role in mediating the underlying mechanism for cocaine craving. These findings have important clinical implications for understanding the genetic basis of variability underlying cue-reactivity, for identifying patients for optimally targeted pharmacotherapy, and more generally for expanding the potential role of cue-exposure paradigms as a clinical tool in future research and service delivery.

*This work was supported by a grant from NIH-NIDA (grant # DA020434-01).

Disclosure: D. Smelson: None. L. Yu: None. S. Buysek: None. G. Gonzalez: None. D. Kalman: None. D. Ziedonis: None.

100. Effects of In Utero Exposure to Psychotropic Medications on DNA Methylation in Neonates

Alicia Smith*, Karen Conneely, Varun Kilaru, James Schroeder, Kimberly Wardlaw, Bettina Knight, Joseph Cubells, Donald Newport, Zachary Stowe, Patricia Brennan

Emory University SOM, Atlanta, GA

Background: There is sparse neurobiological data on the neonates exposed to psychotropic medication in utero. This is remarkable given the level of medication exposure during significant windows of brain plasticity. All psychotropic medications used to date readily cross the placenta, enter into fetal circulation, and most certainly penetrate the fetal blood brain barrier. Furthermore, the ontogeny of neurotransmitter systems that are targets of the major classes of psychotherapeutic drugs, including serotonin, dopamine, glutamate, GABA and norepinephrine, predominantly occurs during the perinatal period in humans. Advances in medical genetics have underscored the contribution of both environmental and genetic factors in establishing developmental trajectories and, more recently, the potential importance of epigenetic alterations, such as DNA methylation, in predicting neurodevelopmental outcomes. No previous study has examined DNA methylation changes associated with prenatal psychotropic exposures.

Methods: To fill this important gap in the literature, we evaluated methylation patterns in DNA extracted from the umbilical cord blood of 257 neonates at delivery whose mothers received pharmacological treatment during pregnancy, comparing the neonates of women with laboratory confirmed and quantified fetal exposure to a) antipsychotic ($N = 40$), b) anti-epileptic ($N = 66$), and c) antidepressant ($N = 107$) medications during their pregnancy compared to d) controls whose mothers did not take psychotropic medications ($N = 34$). Genomic DNA was interrogated across 27,578 CpG sites representing 14,475 genes using the Illumina HumanMethylation27 BeadChip. For each CpG, we assessed the association of each β value with medication use using a linear mixed model that included random effects to adjust for potential chip effects and included gender, race and estimated gestational age (EGA) as covariates.

Results: We observed >100 CpG sites in 92 genes associated with race ($7.66E-33 < p < 1.74E-6$) and 6 CpG sites in 6 genes associated with EGA ($9.50E-09 < p < 1.59E-06$) at a level consistent with experiment-wide

criteria. In contrast to our hypotheses, there were no global or gene-specific associations between any medication class exposure and changes in DNA methylation that met criteria for experiment-wide, Bonferroni-corrected statistical significance ($p < 1.81E-6$). Across the genome, only 1027 (3.7%) CpG sites were nominally associated with exposure to antipsychotic medications ($3.98E-6 < p < 0.05$). Further, 931 (3.4%) CpG sites were nominally associated with exposure to anti-epileptics medications ($1.17E-4 < p < 0.05$), and 1552 (5.6%) CpG sites were nominally associated with exposure to antipsychotic medications ($1.41E-5 < p < 0.05$).

Discussion: Overall these findings suggest no evidence for an impact of maternal prenatal psychotropic exposure on infant DNA methylation patterns. Potential interpretations of these findings include the following: a) moderating variables such as the gestational timing of exposure warrants examination, b) inherent changes in DNA methylation are smaller than could be assessed in this study, or c) the developmental trajectory prenatal methylation patterns are resilient to environmental challenges. Expanding the extant knowledge on the impact of prenatal exposure to maternal psychotropic medications on the full spectrum of fetal development is an important component in clinical decision-making. This work is supported by RC1 MH088609 (NIH to AKS and PB).

Disclosure: A. Smith: Part 4; Schering Plough. K. Conneely: None. V. Kilaru: None. J. Schroeder: None. K. Wardlaw: None. B. Knight: None. J. Cubells: Roche Pharmaceuticals, Schering Plough. D. Newport: None. Z. Stowe: None. P. Brennan: None.

101. Tryptophan Hydroxylase 2 (TPH2) Gene Variants And Clozapine Treatment Response

Renan Souza*, Jeffrey Lieberman, Steven Potkin, Herbert Meltzer, James Kennedy

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

Background: Antipsychotics drugs are the best means to manage schizophrenia symptomatology but there is a considerably high interindividual variability in treatment response and side-effect manifestation during antipsychotic therapy. Previous findings have shown that at least part of the difference in the treatment outcome of schizophrenia patients may be due to genetic variants. Tryptophan hydroxylase (TPH) gene encodes the rate-limiting enzyme in the serotonin biosynthesis. TPH has two isoforms known as TPH1 and TPH2. The TPH2 isoform is mainly expressed in the central nervous system and peripheral serotonergic neurons. The influence of TPH2 genetic variants in antipsychotic response remains unknown. Here, we evaluated whether TPH2 polymorphisms were associated with clozapine treatment response in treatment-refractory schizophrenia subjects.

Methods: We genotyped eight genetic variants in 208 schizophrenia patients who were refractory to typical antipsychotics. Treatment response was evaluated prospectively after six months treatment with clozapine. Treatment response was assessed with the Brief Psychiatric Rating Scale.

Results: Our results indicated that none of the eight TPH2 polymorphisms (rs11178997, rs10784941, rs1386494, rs2171363, rs4760816, rs1386486, rs1487280 and rs1872824) were associated with treatment response in our sample.

Discussion: Previous results have shown no association of TPH1 polymorphism with treatment response. Our results also indicate that possible changes in serotonin synthesis generated by polymorphisms in the TPH2 gene are not associated with clozapine treatment outcome. Nevertheless, genetic variants in other components of the serotonin system have shown association with treatment response (e.g. serotonin receptor 2A - HTR2A). Our results should be read with caution prior independent replication.

Disclosure: R. Souza: None. J. Lieberman: None. S. Potkin: None. H. Meltzer: None. J. Kennedy: Part 1; Glaxo Smith Kline.

102. Circadian Timing Phenotypes in Pedigrees Segregating Bipolar Disorder

Patricia St. Clair*, Se-Joo Kim, Carrie Bearden, Rita Cantor, Susan Service, Ileana Aldana, Gabriel Macaya, Jorge Ospina, Carlos Lopez-Jaramillo, Javier Escobar, Andres Ruiz-Linares, Joseph S. Takahashi, Victor Reus, Nelson Freimer, Costa Rica-Colombia Consortium for Genetic Investigation of Bipolar Endophenotypes

University of California, Los Angeles, Los Angeles, CA

Background: The limited success of efforts to identify loci predisposing to bipolar disorder (BPD) has stimulated efforts to identify BPD-related endophenotypes. It is hypothesized that such traits may better reflect the biology underlying BPD than the clinical diagnosis itself, and therefore may be more straightforward to genetically map. Circadian rhythm and sleep-wake disturbances are well-established core characteristics of BPD. We use actigraphy and the Munich Chronotype Questionnaire (MCTQ) to develop quantitative circadian phenotypes of rest and activity, which we evaluate for familial aggregation and correlation with the BDP phenotype in pedigrees consisting of multiple bipolar type one (BP-I) affected individuals and their first degree relatives, drawn from two related genetically isolated populations.

Methods: We have collected circadian phenotypes from euthymic BP-I subjects and their relatives from seven large multi-generational pedigrees from the Central Valley of Costa Rica and Antioquia, Colombia. Data collection is ongoing; the results presented here were generated from the first 268 individuals assayed (out of a total planned sample of 800). The MCTQ provides self-reports of usual sleep and wake times on work and free days and self-rated chronotype (from extremely early to extremely late). We use these reports to measure the mid-point of sleep on free days (MSF), with adjustments for sleep debt, age, sex, and country. Actigraphic data were collected in one minute epochs over 14 days using Spectrum devices (Phillips Respironics) and Actiware® software was used to estimate times and summary measures of rest-activity. We characterize circadian timing using midpoint of estimated night-time sleep and acrophase (time of peak activity) from cosinor analysis of raw activity data, normalized by device. All phenotypes were adjusted for age, sex, and country covariates when appropriate. We evaluate phenotypes for familiality, using Fcor (S.A.G.E.: Statistical Analysis for Genetic Epidemiology), and for correlation with BP-I. Fcor assesses familiality between parents and offspring (P-O) and among siblings. Covariate adjustments and cosinor analysis were done using SAS (Cary, NC).

Results: The sample for this analysis includes actigraphic data from 98 subjects (27 BP-I and 71 of their relatives) and MCTQ data from 217 subjects (51 BP-I, 166 relatives). MCTQ chronotype and MSF correlated significantly ($r = 0.36-0.70$) with analogous phenotypes from actigraphy, showing consistency of these measures. The most heritable actigraphy phenotypes are midpoint of sleep and acrophase, with sibling correlations of 0.467 ($p = 0.016$) and 0.502 ($p = 0.011$), and parent-offspring (P-O) correlations of 0.668 ($p < 0.001$) and 0.356 (ns), respectively. The MCTQ MSF had a modest but nominally significant P-O correlation of 0.180 ($p = 0.042$), and a non-significant sibling correlation. Self-rated chronotype showed a suggestive but small P-O correlation of 0.191 ($p = 0.069$) as did the 'extreme early' phenotype with P-O and sibling correlations of 0.188 ($p = 0.079$) and 0.163 ($p = 0.082$), respectively. On average, BP-I subjects and their relatives report a normal chronotype, but in our current sample, BP-I subjects tend to have later timing for peak activity and mid-sleep behavior. Comparison of means between BP-I and relatives for acrophase (0.46 ± 1.29 vs -0.18 ± 1.5), actigraphic mid-sleep (11.2 ± 81.8 vs -4.2 ± 64.8), and MCTQ MSF (0.097 ± 1.23 vs -0.034 ± 0.90) show a consistent pattern of later times for BP-I subjects.

Discussion: To our knowledge, this is the first analysis of actigraphy phenotypes and self-reported chronotype that examines both familiality and correlation with BP-I. The results from our pilot sample

suggest that circadian phenotypes may be useful intermediate traits for genetic studies of BPD. Limitations of this analysis include the relatively small sample size, however future data collection will increase the sample by several fold. Our preliminary comparisons of BP-I patients with their relatives do not account for family relationships; other tests of association will be conducted when the sample is larger. Nevertheless, initial results indicate that circadian phenotypes of rest and activity timing show promise for further study as quantitative traits associated with BPD.

Disclosure: P. St. Clair: None. S. Kim: None. C. Bearden: None. R. Cantor: None. S. Service: None. I. Aldana: None. G. Macaya: None. J. Ospina: None. C. Lopez-Jaramillo: Part 1; Dr López-Jaramillo has participated in speakers/advisory boards for Abbott, AstraZeneca, GlaxoSmithKline, and Janssen. Part 4; Dr López-Jaramillo has received research support from AstraZeneca, Lundbeck, and Wyeth. J. Escobar: None. A. Ruiz-Linares: None. J. Takahashi: Part 1; Dr. Takahashi is cofounder and scientific advisory board member of ReSet Therapeutics Inc. V. Reus: None. N. Freimer: None.

103. Meta-Analysis Of The Glutamate Transporter Gene SLC1A1 In Obsessive-Compulsive Disorder

Evelyn Stewart*, Catherine Mayerfeld, Jacquelyn Crane, Jesen Fagerness, Colm O'Dushlaine

Massachusetts General Hospital, Boston, MA

Background: Obsessive-Compulsive Disorder is a severe psychiatric disorder affecting 1-3% of the population. The complex genetic nature of the disorder is not well understood, and despite numerous family, twin, and segregation analyses indicating the heritability of OCD, candidate gene studies have not been consistently replicated. Of these, the glutamate transporter gene SLC1A1 is the best replicated and is also a strong functional candidate, as various lines of evidence suggest that abnormal glutamatergic neurotransmission is involved in OCD pathogenesis. However, while positive associations between OCD and SLC1A1 have been consistently reported, these have involved different SLC1A1-related SNPs.

Methods: This study combined available SNP data from published and unpublished OCD SLC1A1 candidate gene studies. Selection of SNPs for inclusion in this study was based on whether a significant association, either as a single marker or as part of a haplotype, had been previously reported for the SNP and whether data was available from at least one additional study sample. Original, pre-QC data from all the sites were cleaned using standard QC measures. A mega-analysis of family-based association data was performed using FBAT, while single marker analyses for the case-control sample were performed in Plink. For the meta-analysis, association results from these two cohorts were combined using weighted z-scores. Each single-marker analysis was weighted individually, using the Genetic Power Calculator to determine the equivalent effective sample size in a case-control study with case-to-control ratio of 1.

Results: Samples included in these analyses were incorporated from one case-control study [Wendland et al., 2009] and four family-based studies [Arnold et al., 2006; Dickel et al., 2006; Stewart et al., 2007; Shugart et al., 2009]. Unpublished family-based data from the OCD Mini-Collaborative was also included. The total sample size was 4280 individuals, including 1068 pedigrees (3340 persons from 820 nuclear families) and 306 cases with 634 ethnically- and sex-matched controls. Among OCD-affected individuals, 44% (N=920) were male. Nine SNPs met criteria for inclusion, and because not all of these SNPs had been genotyped in all of the studies, each SNP was weighted and analyzed individually. Of these nine SNPs, all were found to be positively associated with OCD in at least one of the studies included in our study, though three of these SNPs were significant only in haplotype analyses. Most of these reported single-marker and haplotype associations were found only in males after samples had been stratified by gender. In the meta-analysis we performed, none of these SNPs retained a significant association signal.

($p < .05$), even before correction for multiple testing. When our sample was stratified by proband gender and the SNPs were analyzed in males only, one SNP (rs12682807) had a p-value of 0.035243 in the merged family analysis, which was the final p-value for that SNP, as it had not been genotyped in the case-control cohort. However, this association does not survive even the most liberal of multiple testing corrections.

Discussion: The aim of this study was to provide clarification regarding the role of SLC1A1 as a candidate gene. Although multiple studies have reported significant association of SLC1A1 and OCD phenotype, the significance was lost when these studies were combined. The smallest of these original studies contained 66 families [Stewart et al., 2007] and the largest contained 378 families [Shugart et al., 2009]. The combined sample for our meta-analysis included 1068 pedigrees and 306 cases with 634 controls. Our results underscore the need for much larger sample sizes and demonstrate the limitations of the candidate gene approach to gene discovery. The atheoretical nature of Genome-Wide Association Studies makes them indispensable in the ongoing search for OCD-related genes.

Disclosure: E. Stewart: None. C. Mayerfeld: None. J. Crane: None. J. Fagerness: None. C. O'Dushlaine: None.

104. Association of NRG1 Genetic Variants with Psychosis in Alzheimer Disease and with Brain Expression of Neuregulin Isoforms

Marianne Seney, Bernie Devlin, Robert Ferrell, Lambertus Klei, Oscar Lopez, Howard Seltman, Etienne Sibille, Robert Sweet*

University of Pittsburgh and VAPHS, Pittsburgh, PA

Background: Neuregulin 1 (NRG1) has been identified as a potential risk gene for psychosis based on evidence of linkage and association with schizophrenia. Similarly, we previously reported linkage and association of NRG1 in late onset Alzheimer Disease with psychosis using a small set of genetic markers (Go et al., 2005). We therefore undertook to more thoroughly evaluate genetic variation in NRG1 for association with psychosis in individuals with AD.

Methods: 867 unrelated subjects were diagnosed with Probable or Possible AD and characterized for psychosis at the U. Pitt ADRC. Tag SNPs ($r^2 < 0.8$) were identified from HapMap using HClust and genotyped using the Illumina Golden Gate assay. After editing for QC, 218 SNPs were analyzed. We used a gene-based test of association designed to maximize power for detecting effects when present within haplotype blocks (Roeder et al., 2005).

Results: We found evidence of a region of significant association within NRG1 with psychosis in AD [$p = 0.025$]. The region extended from rs12550308 (31933352 bp, conditional logit analysis controlling for ancestry $p = 0.0014$) to rs10954822 (32026808 bp, conditional logit analysis controlling for ancestry $p = 0.011$). Bioinformatic review of this region indicated it includes a non-coding sense mRNA (BC037250) likely to regulate transcription within NRG1, and is immediately upstream of the transcription initiation site for a novel NRG1 isoform (Type V). To further investigate the possible relationship of rs12550308 with NRG1 expression, we genotyped rs12550308 in a cohort of individuals in whom NRG1 expression in anterior cingulate cortex gray matter had been previously quantified using Affymetrix Human Genome U122Plus-2.0 (Sibille et al., 2009). Homozygosity for the minor (risk) allele of rs12550308 was associated with increased expression of probes ILMN_1824554 and ILMN_1842205 which target Type V mRNA. rs12550308 genotype was not associated with expression of probes targeting other NRG1 isoforms, nor with probe ILMN_1862563 targeting BC037250. The association of rs12550308 with NRG1 Type V mRNA expression was confirmed by qPCR.

Discussion: We provide evidence that genetic variation in NRG1 is associated with psychosis in AD, and have identified a potential unique mechanism via effects on transcription of NRG1 Type V. Clearly replication of the association of genetic variation within this region of NRG1 in other AD cohorts characterized for psychosis is necessary.

If confirmed, further efforts should be directed towards the identification of liability alleles located within the region of increased association, and towards evaluating their effects on NRG1 expression in brain of individuals with AD + P.

Disclosure: M. Seney: None. B. Devlin: None. R. Ferrell: None. L. Klei: None. O. Lopez: None. H. Seltman: None. E. Sibille: None. R. Sweet: None.

105. Examining the Association Between the BDNF Gene and a Combination Phenotype of Hoarding and Obesity

Kiara Timpano*, Norman B. Schmidt, Michael Wheaton, Jens Wendland, Dennis Murphy

University of Miami, Coral Gables, FL

Background: Across recent animal and human genetic investigations, the gene coding for the brain derived neurotrophic factor (BDNF) has emerged as an interesting candidate for multiple brain and brain disorder-related phenomena. Studies using gene-targeted murine models have found that Bdnf variation is linked with memory impairment, greater avoidance, greater anxiety, aggression, and obesity. Parallel findings have emerged from human investigations for the Val66Met BDNF SNP. The primary aim of the present investigation was to examine the relationship between the BDNF variant Val66Met and two phenotypes: compulsive hoarding as a symptom dimension of obsessive-compulsive disorder (OCD), and body mass index (BMI) levels. BDNF has been linked with energy metabolism in both animals and humans, and from an evolutionary perspective, both internal fat storage and the hoarding of food stores are important strategies for managing energy demands. There is some support linking greater BMI levels to both the BDNF Val66Met variant and hoarding; however, the relationship between BDNF and hoarding has not been examined. Although a handful of past investigations considered the relationship between BDNF and OCD within the context of symptom dimensions, adequate assessments of hoarding were not included, and may therefore have masked any associations.

Methods: In the present report we examined the BDNF gene in a large ($N = 301$) clinical sample of probands with OCD. Participants were classified as hoarding and non-hoarding using a strict, multi-measure grouping approach. Approximately 25% of the sample exhibited clinically significant hoarding symptoms. The BDNF Val66Met polymorphism (dbSNP rs6265) was genotyped by a 5'-exonuclease assay. In line with previous reports and evidence that the Met allele may be functionally dominant, we compared the Val/Val group to Met allele carriers.

Results: The functional BDNF gene SNP Val66Met was found to be strongly associated with both hoarding and obesity. The Val/Val genotype was linked with more severe hoarding behaviors ($\beta = .17$, $t(198) = 2.44$, $p < .02$) and hoarding classification ($OR = 2.2$, 95% CI = 1/21 - 3.96, $p < .01$), as well as greater BMI levels ($\beta = .15$, $t(254) = 2.34$, $p < .02$) and obesity classification ($OR = 2.3$, 95% CI = 1.24 - 4.40, $p < .01$). Hoarding status was also associated with greater BMI scores, with individuals in the hoarding group being far more likely to be classified as obese compared to the non-hoarding group ($OR = 3.29$, 95% CI = 1.77 - 6.12, $p < .001$). BDNF Val/Val females ($N = 179$) had a much greater relative risk for obesity ($OR = 3.08$, $p < .01$) and hoarding classification ($OR = 2.1$, $p < .05$), compared to males.

Discussion: The present report is the first to examine associations between BDNF, hoarding, and BMI. Our finding that the relationship between hoarding and greater BMI was the strongest for those individuals with the Val/Val genotype may provide a distinct avenue through which hoarding and BMI could be related. For example, within the animal literature, serotonin transporter [serT]-deficient mice and those interbred with Bdnf-deficient mice display greater stress responses, anxiety responses and obesity (Ren-Patterson and others 2005). That is, the joint associations between BDNF, hoarding, and BMI may in part explain a genetic relationship between hoarding and BMI. These findings suggest a three-way gene x body weight x

psychopathology interaction wherein a primitive, survival “thrifty gene” strategy may be conserved and represented in a subgroup of humans manifesting severe hoarding symptoms.

Disclosure: K. Timpano: None. N. Schmidt: None. M. Wheaton: None. J. Wendland: None. D. Murphy: None.

106. BDNF Val/Met Polymorphism Interacts with Early-Life Stress to Predict HPA Axis Function

Audrey Tyrka*, Lawrence Price, Joel Gelernter, Chris Walters, Linda Carpenter

Brown University/Butler Hospital, Providence, RI

Background: Brain-derived neurotrophic factor (BDNF) is a nerve growth factor that is reduced in response to stress and is implicated in the pathogenesis of major depression. The functional Val66Met BDNF polymorphism has been shown to interact with early-life stress to predict adult depression. A large body of literature documents altered HPA axis function with both depression and early life stress. We have previously found that adults with a history of childhood maltreatment have attenuated cortisol responses to psychosocial and neurobiological challenge. This study was conducted to determine whether this BDNF polymorphism influences the neuroendocrine response to early-life stress.

Methods: Healthy adults ($N=164$) provided blood samples for DNA extraction and genotyping of the BDNF val66met polymorphism and completed the Childhood Trauma Questionnaire. A standardized neuroendocrine challenge test, the dexamethasone/corticotropin-releasing hormone (Dex/CRH) test, was administered. A repeated measures general linear model controlling for age, sex, and diagnosis of depressive disorder, tested effects of BDNF Val66Met genotype and childhood maltreatment on cortisol response to the Dex/CRH test.

Results: There was an interaction of sex x genotype ($p<.05$) such that in males, Met carriers had lower cortisol responses to the test, whereas in females genotype did not determine cortisol responses. There was also an interaction of childhood maltreatment x genotype ($p<.05$) such that among adults with a history of childhood maltreatment, Met carriers had lower cortisol concentrations, but in the absence of maltreatment there was no effect of genotype.

Discussion: These findings suggest that the BDNF val66met polymorphism influences HPA axis function differentially in response to childhood maltreatment, and suggests that Met carriers are more sensitive to the neuroendocrine effects of early-life stress. Implications for the pathophysiology of stress-related disorders will be discussed.

Disclosure: A. Tyrka: Part 1; Lundbeck, Takeda. Part 4; Medtronic, Cyberonics, Neuronetics, Sepracor, UCB Pharma, Pfizer. L. Price: Part 1; Sepracor, Cyberonics, Medtronic, Neuronetics, MD Conferences/Psychiatry Review Course, Abbott, Gerson Lehrman, Wiley, Springer. Part 2; Wiley. Part 4; Sepracor, Cyberonics, Medtronic, Neuronetics. J. Gelernter: None. C. Walters: None. L. Carpenter: Part 1; Abbott, Cyberonics, Novartis, Wyeth, AstraZeneca, Neuronetics. Part 2; Neuronetics. Part 4; NARSAD, UCB Pharma, Sepracor, Cyberonics, Medtronic, Neuronetics.

107. A SNP Array Study Identifies Reelin and Phosphodiesterase Genes That Influence Cerebral Cortical Morphology in Schizophrenia

Thomas Wassink*, Beng-Choon Ho, Steven Ziebell, Eric Epping, Nancy Andreasen

University of Iowa Carver College of Medicine, Iowa City, IA

Background: Schizophrenia is a highly heritable neurodevelopmental disorder characterized by diffuse structural abnormalities of the cerebral cortex. Normal brain development, and thus adult brain morphology, is itself under substantial genetic control. Identification of genetic factors that contribute to variability of brain morphology in schizophrenia may 1) identify risk factors for the disorder itself, 2) illuminate other aspects of phenotypic variability, and 3) indicate potential targets for more efficacious treatments.

Methods: We genotyped 28,940 SNPs from 1204 genes in 250 individuals with schizophrenia, most of whom had undergone structural magnetic resonance imaging (MRI). The selected genes included: 1) genes previously associated with schizophrenia through linkage, association, or chromosomal abnormalities; 2) brain developmental genes; 3) synaptic genes; and 4) neurotransmitter pathway genes. Tagged SNPs were chosen to provide dense linkage disequilibrium coverage of the genes and were placed on a custom Illumina SNP microarray chip. Participants all had a DSM-III-R or DSM-IV diagnosis of schizophrenia as determined by a semi-structured interview and a multi-disciplinary team assessment, and 235 participants had undergone high-resolution multi-modal brain MR imaging. The images included T1 and PD/T2 scans, and were processed using BRAINS2 software that automatically generated gray and white matter volumes for the four cerebral lobes, and cerebrospinal fluid (CSF) volume for the lateral ventricles. Statistical analysis was performed in PLINK using a linear regression-based analysis of covariance (ANCOVA). Gender and age were included as covariates, volumes for the nine brain structures were the dependent measures, and genotype was the predictor. Tests were performed using both the “additive” and “genotypic” options and a Bonferroni corrected p-value of $0.05/28940 = 1.7 \times 10^{-6}$. For genes containing at least five SNPs with at least two having $p < 0.001$, we used PLINK to perform a gene-based permutation test (10,000 permutations) to assess the combined influence of all SNPs from those genes on the outcome measures.

Results: No single SNP produced a significant effect on cerebral cortical volumes when using the Bonferroni correction for multiple testing. Approximately 20 genes met criteria for the gene-based test. Of these, genetic variation in Reelin (*RLN*) produced the strongest evidence for influencing brain structure volumes, including frontal gray ($p = 0.001$), frontal white ($p = 0.001$), parietal gray ($p = 0.0004$), and parietal white ($p < 0.0001$). The Reelin SNPs of strongest effect were spread out over approximately 50 kb of the 5' end of the gene and clustered into three LD based groups. Other genes implicated by the permutation analyses included *PDE4B*, *PDE8B*, *PER3*, and *TGFBR2*.

Discussion: Reelin is a brain developmental protein that influences cortical neuronal migration and the development of cortical lamination. Some evidence suggests the involvement of Reelin in schizophrenia through both genetic and epigenetic mechanisms. We now bring these two trains of thought together by showing that genetic variation in *RLN* may influence brain morphology in individuals with schizophrenia. We also implicate two phosphodiesterases, a circadian rhythm protein, and a protein kinase. Such insight may better illuminate how the brain pathology of schizophrenia actually arises, which could in turn inform understanding of symptom progression and of treatment. Future directions include testing the genetic variation from the implicated genes in both control samples and additional samples of individuals with schizophrenia.

Disclosure: T. Wassink: Part 1; Ortho McNeil Janssen Scientific Affairs LLC. Part 4; Ortho McNeil Janssen Scientific Affairs LLC. B. Ho: Part 1; Ortho McNeil Janssen Scientific Affairs LLC. Part 4; Ortho McNeil Janssen Scientific Affairs LLC. S. Ziebell: Part 1; Ortho McNeil Janssen Scientific Affairs LLC. Part 4; Ortho McNeil Janssen Scientific Affairs LLC. E. Epping: None. N. Andreasen: Part 1; Ortho McNeil Janssen Scientific Affairs LLC. Part 4; Ortho McNeil Janssen Scientific Affairs LLC.

108. Correlation Between DAT1 Polymorphism, Dopamine Transporter Density, and Eye Tracking: A Combined Genetic and PET Imaging Study

Ikwunga Wonodi*, Melin Vranesic, Henry Holcomb, Laura Rowland, Elliot Hong, Dean Wong, Gunvant Thaker, Zsolt Szabo

Maryland Psychiatric Research Center, University of Maryland School of Medicine, Baltimore, MD

Background: We have previously demonstrated a paradoxical association between a functional variable number of tandem repeats (VNTR) polymorphism in DAT1 gene and predictive pursuit eye movement

deficits in schizophrenia but normal predictive pursuit in healthy controls. Subsequently, we demonstrated differential effects of the DAT1 VNTR 10-repeat allele vs. non-10 repeat allele (i.e., 10/10 vs. non-10/10) genotype on DAT1 mRNA expression in post-mortem frontal eye field (FEF) samples in schizophrenia compared to control donor tissue. DAT1 10/10 genotype correlated with reduced transcript [i.e., increased synaptic dopamine] in normal controls, but had the opposite effect [increased transcript or putatively, reduced synaptic dopamine] in schizophrenia. Neuroimaging data from monkeys and humans have identified the FEF as a key cortical region associated with smooth pursuit eye movements. The current data includes a follow-up study where we aim to connect our predictive pursuit findings with DAT1 genotype using an *in vivo* measurement of gene expression. Overlapping components of the predictive pursuit circuitry are modulated by dopaminergic neurotransmission in the FEF and substantia nigra.

Methods: Postmortem sample: DAT1 mRNA expression in 32 post-mortem FEF tissue samples (16 schizophrenia cases closely matched with 16 controls). Clinical sample: six schizophrenia and 5 control participants have been accrued in the current ongoing study. Subjects were recruited based on diagnosis, DAT1 genotype, and predictive pursuit measures. All subjects had MRI and PET scans. MRI was used to co-register PET images. Dopamine transporter density was measured with [¹¹C]WIN35,428 binding during PET. Radioligand binding was quantified by the standardized uptake value ratio SUVR widely used for radioligands with tight receptor binding like [¹¹C]WIN.

Results: Postmortem, DAT1 10/10 genotype was associated with significantly reduced mRNA expression in schizophrenia FEF ($p < 0.05$), but higher mRNA expression in FEF tissue from schizophrenia donors ($p = 0.08$). In the clinical sample, examination of the effects of diagnosis group by DAT1 genotype on transporter binding density showed a similar trend to postmortem mRNA expression findings.

Discussion: These results suggest a differential effect of DAT1 genotype on DAT1 gene expression in schizophrenia FEF, and a trend towards a similar association measured *in vivo* during PET imaging with the dopamine transporter receptor-specific radioligand, [¹¹C]WIN. However, the clinical PET imaging sample size is small. Subject recruitment is ongoing.

Disclosure: I. Wonodi: None. M. Vranesic: None. H. Holcomb: None. L. Rowland: None. E. Hong: None. D. Wong: None. G. Thaker: None. Z. Szabo: None.

109. Meta-analysis of Genetic Variation in DTNBP1 and General Cognitive Ability

Jianping (Jimmy) Zhang*, Katherine Burdick, Todd Lencz, Anil Malhotra

The Zucker Hillside Hospital, Glen Oaks, NY

Background: The human dystrobrevin-binding protein 1 (DTNBP1) gene has been linked to risk for schizophrenia. Recent studies indicate that several single nucleotide polymorphisms (SNPs) in the DTNBP1 gene may also influence general cognitive ability in both schizophrenic patients and healthy controls. We examined the relationship between DTNBP1 SNPs and general cognitive ability in non-psychiatric healthy samples via meta-analysis.

Methods: Medline search (12/31/2009) yielded 11 articles examining DTNBP1 variation and general cognitive ability, of which 8 studies had data available encompassing 10 independent cohorts (total $n = 7,592$). The phenotype was defined as either the first principal component score from multiple neuropsychological tests (Spearman's ρ) or full scale IQ. Meta-analyses were conducted for 9 SNPs for which cognitive data were available from at least 3 cohorts. For each SNP in each cohort, effect size (ES) was computed between major allele homozygotes and minor allele carriers; ES was then pooled across studies using a random effect model.

Results: Pooled ES's from 2 of the 9 SNPs (rs1018381 and rs2619522) were -0.123 and -0.083, $p < 0.01$, respectively, suggesting that the minor allele carriers of these SNPs had lower cognitive ability scores than the major allele homozygotes. Results remained significant after examining heterogeneity among samples and potential publication biases. Other SNPs did not show significant effects on general cognitive ability.

Discussion: Genetic variation in DTNBP1 modestly influences general cognitive ability. Further studies are needed to elucidate the biological mechanisms that may account for this relationship.

Disclosure: J. Zhang: None. K. Burdick: Part 1; Merck. T. Lencz: Eli Lilly, Merck, Clinical Data Inc., GoldenHelix, Inc., Guidepoint Global, Cowen & Co. A. Malhotra: Wyeth, PGx Health, Eli Lilly, Schering-Plough/Merck, Arbor Scientia (Sepracor Inc.). Part 4; Eli Lilly.

110. Platelet Protein Kinase C and Brain-Derived Neurotrophic Factor Levels Are Reduced in Borderline Personality Disorder Patients Compared to Healthy Volunteers

Harold W Koenigsberg*, Peixiong Yuan, Larry J Siever, George A Diaz, Christine Dorantes, Stephanie Guerreri, Sarah Jo Mayson, Antonia S New, Marianne Goodman, Husseini Manji

Mount Sinai School of Medicine, Bronx, NY

Background: Borderline personality disorder (BPD) is a prevalent and enduring psychiatric condition which affects 2 to 5.9% of the population. It is characterized by abrupt mood swings, intense anger and depression, unstable interpersonal relationships, impulsive self-destructive behavior and a suicide rate of approximately 10%. The disorder is difficult to treat and its response to pharmacotherapy is modest. Possible underlying neurochemical dysregulations in BPD have not been well explored. Protein kinase C (PKC) and brain-derived neurotrophic factor (BDNF) both play important roles in an array of neural processes including neurotransmission, intracellular signal transduction, regulation of neuronal plasticity and gene expression. Dysregulations in these substances have been implicated in both bipolar disorder and depression, but they have not been examined in BPD to our knowledge. The prevalence of mood dysregulation in BPD, the high comorbidity of BPD and depression, and the proposal that BPD exists on a continuum with bipolar disorder suggest that PKC and BDNF dysregulations may contribute to BPD. Study of these systems in BPD can help us to better understand the mechanisms of mood disturbance in BPD and its relationship to both depression and bipolar disorder.

Methods: Platelets were isolated from blood obtained from 26 medication-free BPD patients and 17 healthy control (HC) subjects. PKC-alpha (PKCa), phosphorylated-PKC-alpha (p-PKCa) and BDNF were measured in platelet homogenates by immunoblotting. Group differences were tested using a generalized linear model, covarying for age.

Results: In the entire sample, p-PKCa activity was lower in BPD patients compared to healthy controls (BPD: 1.65 ± 0.26 ; HC: 2.22 ± 0.32 ; $F(1,39) = 5.12$, $p = 0.03$). There were no group differences in PKCa or BDNF levels. In the males, PKCa and BDNF levels were also lower in BPD patients compared to HC's (PKCa: BPD: 8.71 ± 1.62 vs. HC: 16.21 ± 2.07 ; $F(1, 26) = 12.12$, $p = .002$; BDNF: BPD: 4.60 ± 1.45 vs. HC: 8.62 ± 1.86 ; $F(1,26) = 7.14$; $p = 0.01$).

Discussion: Activation of PKCa (p-PKCa) is reduced in BPD patients relative to healthy volunteers and in males PKCa and BDNF expression is reduced in BPD patients compared to controls. This is the first report of PKC and BDNF activity in BPD to our knowledge and calls for replication. These findings are consistent with altered PKC and BDNF activity in a range of neuropsychiatric conditions including bipolar disorder, depression and in suicide.

Disclosure: H. Koenigsberg: None. P. Yuan: None. L. Siever: None. G. Diaz: None. C. Dorantes: None. S. Guerreri: None. S. Mayson: None. A. New: None. M. Goodman: None. H. Manji: Part 5; Dr Manji is currently a full time employee of Johnson and Johnson Pharmaceuticals.

111. Implications of Entrainment of Blind Free-Runners to Low-Dose Melatonin for the Phase Shift Hypothesis (PSH) for Winter, Non-Seasonal and Summer Depression and the Maternal Ontogenetic Melatonin (MOM) Hypothesis for Early Disorders

Alfred Lewy*

Oregon Health & Science University, Portland, OR

Background: The ability to be entrained directly by the 24-hour light/dark cycle does not occur in humans until 12 months after conception; however, melatonin receptors are functional by at least the third trimester. A function of melatonin is for the mother to have a chronobiologic effect on the developing baby via placental transfer and breast milk (see 1 for further references). About 1-5% of people are low melatonin producers. It is therefore critical that low levels of melatonin, even in breast milk, are sufficient for entrainment. Totally blind people are a group of subjects ideally suited for testing the chronobiologic effects of low doses of melatonin.

Methods: 1) Ten totally blind free-running adults were given melatonin (in the evening); the dose was stepped up or down, in order to detect the lowest entraining dose. Doses were between 0.02 and 0.3 mg, which were within the physiological range. In a follow-up study, a subject was given 0.01 mg, which produces levels of melatonin lower than made by most people at night. Data are reported here for the first time for all 11 subjects. 2) A Portland, Oregon patient, with recurrent summer depression (and winter hypomania) has been treated with 0.3 mg of melatonin (taken in the afternoon so as to prevent a phase-delay in circadian rhythms with respect to the sleep/wake cycle) since April 1, 2010, at least 2 months before he was expected to become depressed. When initiated after the patient had become depressed the prior summer, this treatment was not effective, nor were any of the numerous trials of medication combinations typically used to treat bipolar II (and unipolar) depression.

Results: 1) All blind subjects entrained to a daily dose of melatonin. The resulting dose-response curve was log-linear and statistically significant [$r(9) = 0.67$, $p = 0.02$]. 2) As of Aug. 25, 2010, 2-3 months after the usual start of his seasonal depression, he remains euthymic.

Discussion: 1) Since free-running blind people can be entrained to doses of melatonin as low as 0.01 mg, then endogenous levels of melatonin in the fetus - and even in the suckling infant - through maternal transfer in the blood and breast milk, respectively, could be important as a 24-hour timing signal for optimal embryonic, fetal and later development. Low melatonin production in the mother might therefore be a necessary, but not sufficient, cause of autism (1) or other disorders linked to early development (such as schizophrenia), as well as affect subsequent quality of life. Therefore, restoring maternal melatonin levels to normal should be a safe and effective treatment for entraining the fetus and the "fourth trimester" infant to the 24-hour day. The maternal ontogenetic melatonin (MOM) hypothesis can be easily tested by first determining if mothers of afflicted individuals who are low melatonin producers [or carry mutations of the (ASMT) gene that determines the amount of melatonin production] are more prevalent than in the general population. If so, in a prospective study, melatonin can then be safely given to pregnant and nursing low melatonin-producing mothers who are also at risk for giving birth to afflicted children. 2) Although only a single case report, the fact that this patient did not become depressed this year (apparently in response to low-dose melatonin) has several implications. One, phase-delayed (and not phase-advanced) circadian misalignment seems to be the most common biological rhythm abnormality in winter, non-seasonal and now perhaps summer depression. Two, according to the biopsychosocial(environmental) model, clinical efficacy was achieved only when treatment was given to prevent occurrence of depression; once depression occurs, it is understandable that even if the biological precipitant is corrected, the other subsequent causes and manifestations of depression are not easily or quickly reversible. Three, agents that can cause corrective

phase advances (or prevent phase delays, such as morning bright light and low-dose afternoon/evening melatonin, can potentially be used to treat many types of seasonal and non-seasonal depression, as well as other co-morbid disorders. Four, perhaps this patient delayed each summer because his intrinsic period might be < 24 hours (and, therefore, he cues to the later dusk of longer days). Because low-dose melatonin in particular is safe, convenient and inexpensive and because the dim light melatonin onset (DLMO) collected at home or in the sleep lab may become the first useful biological test in psychiatry, all of these factors bode well for low-dose melatonin and the phase shift and MOM hypotheses to have a major impact in clinical medicine 1. Lewy, A.J. *JCEM* (2010): 95(7): 3158-3160.

Disclosure: A. Lewy: Part 2; Servier. Part 3; Servier.

112. Neurosteroids are Altered in Veterans who Sustained a Blast-Related Traumatic Brain Injury (TBI) in Iraq or Afghanistan

Chris Marx*, Jason Kilts, Jennifer Naylor, Lawrence Shampine,

Jennifer Strauss, Larry Tupler

Duke University Medical Center and Durham VA, Durham, NC

Background: Large numbers of Veterans have sustained a blast-related traumatic brain injury (TBI) in Iraq or Afghanistan, but the pathophysiology of this disorder has not been comprehensively elucidated and therapeutic strategies remain limited. TBI is associated with multiple neuropsychiatric sequelae (including PTSD and depression symptoms), and it is also associated with pituitary dysfunction. Further, recent randomized controlled trials in clinical cohorts suggest that the neurosteroid progesterone (a molecule synthesized *de novo* in human brain) may have therapeutic efficacy for moderate to severe TBI. Since neurosteroid changes have been associated with PTSD and depression, and since neurosteroids modulate the hypothalamic-pituitary-adrenal axis, we hypothesized that neurosteroid regulation may be altered following blast-related TBI.

Methods: Neurosteroid levels in serum were quantified in 55 male Veterans who had sustained a blast-related TBI in Iraq or Afghanistan, and in 55 male control Veterans who had served in Iraq or Afghanistan but who had not sustained a blast-related TBI (matched for age, time of blood draw, and smoking status). Gas chromatography/mass spectrometry preceded by high performance liquid chromatography was utilized to quantify the neurosteroids pregnanolone, androsterone, pregnenolone, allopregnenolone, and epiallopregnenolone.

Results: The GABAergic neurosteroid pregnanolone was significantly reduced in Veterans who had sustained a blast-related TBI in Iraq or Afghanistan compared to Veterans who had no TBI history (Mann-Whitney $p = 0.0010$, $n = 55$ per group), as was the GABAergic neurosteroid androsterone (Mann-Whitney $p = 0.0011$, $n = 55$ per group). Pregnenolone also tended to be reduced in Veterans who had sustained a blast-related TBI (Mann-Whitney $p = 0.0769$). Allopregnenolone and epiallopregnenolone levels were not significantly different in Veterans with a history of blast-related TBI compared to those with no history of TBI.

Discussion: Several neurosteroids appear to be reduced in Veterans who sustained a blast-related TBI in Iraq or Afghanistan. It is possible that these changes may reflect a dysregulation in neurosteroids that is precipitated by blast-related TBI, potentially via pituitary injury. Neurosteroid dysregulation post-TBI may thus be a candidate mechanism contributing to neuropsychiatric symptomatology associated with this event. It is also possible that neurosteroid restoration following TBI may represent a logical therapeutic strategy. Future efforts will be required to test these preliminary hypotheses.

Disclosure: C. Marx: Part 1; Patents pending (neurosteroids and derivatives in neuropsychiatric disorders, neurosteroids for lowering cholesterol); no patents issued; no licensing in place. J. Kilts: None. J. Naylor: None. L. Shampine: None. J. Strauss: None. L. Tupler: None.

113. Myoinositol, NAA, and Anxiety in Patients During Nicotine Withdrawal

Graeme Mason*, Irina Esterlis, June Watzl, Jie Wang, Ran Wu, Suchitra Krishnan-Sarin, Robert Makuch, Julie Staley, Stephanie O'Malley

Yale University, New Haven, CT

Background: The nature of tobacco smoking differs between women and men. Women, less sensitive and more tolerant to the pharmacological effects of nicotine, have been reported to exhibit a poorer response to nicotine replacement therapies. Women are also more vulnerable to subsyndromal mood disorders (depression and anxiety) that are linked to chronic smoking. In brain, nicotine binds to nicotinic acetylcholine receptors and initiates release of most major neurotransmitters including γ -aminobutyric acid (GABA). This project used magnetic resonance spectroscopy (MRS) to measure brain GABA, glutamate, NAA, myoinositol, and other compounds in men and women who quit smoking, with the theory that GABA and glutamate levels would be affected in sex-specific manners that might be related to anxiety or depression often observed in women smokers.

Methods: 8 men smokers, 16 men nonsmokers, 8 women smokers, and 18 women smokers were enrolled in the study and proceeded through the protocol to yield data. All smokers were scanned twice, once before quitting and once \sim 10 days after quitting, with women scheduled as close as possible to obtain both scans during the follicular phase of the menstrual cycle. Women nonsmokers were scanned twice during the follicular phase of the menstrual cycle. Men nonsmokers were scanned only once. Abstinence-contingent reinforcement procedures were used to achieve abstinence from smoking. Symptoms of anxiety and depression were assessed with the Spielberger State/Trait Anxiety Scale and the Center for Epidemiological Studies-Depression scale, respectively, on days close to the MRS scans. Scanning was done with a 4 Tesla magnet (Bruker Instruments, Billerica, MA, USA) while performing J-difference editing of GABA, using a surface coil in the occipital cortex to achieve sensitivity in this region where differences in GABA receptor binding have been reported in smokers relative to nonsmokers. Metabolites were quantified relative to tissue water and tissue creatine. Metabolites other than GABA were quantified from the unedited subspectrum.

Results: Overall, women had lower initial glutamate/creatinine levels than the men ($p = 0.011$), but this may be explained by higher levels of creatine relative to tissue water in the women. Smoking did not show a relationship with glutamate or GABA. However, among women, initial levels of NAA and myoinositol were lower for the smokers than the nonsmokers ($p = 0.034$ and 0.025 , respectively). For men who completed both the initial and abstinent scans, myoinositol increased in relation to Trait Anxiety ($p = 0.01$).

Discussion: The lower levels of NAA would be suggest greater neurotoxicity or energetic compromise in women. Lower NAA has been seen in smokers with previous reports of smokers with alcohol dependence (2) and without known comorbidities (3), but not with respect to gender with smoking. The reason for the greater increase in myoinositol in individuals who had greater anxiety is less clear. Elevations of myoinositol are typically associated with neurologic pathology, so it is possible that in this case the stress of the first days of smoking cessation causes an elevation of myoinositol. However, if the elevation is stress-related, it is likely to be temporary, as the brain, body, and vasculature adapt to the healthier, smoke-free condition.

References: 1. Smith MJ, Keel JC et al (1999) Neurology 53: 2069-2072 2. Gazdzinski S, Durazzo T et al (2008) Psychiatry Res 162: 133-145 3. Gallinat J, Schubert F (2007) Pharmacopsychiatry 40: 64-67.

Disclosure: G. Mason: None. I. Esterlis: None. J. Watzl: None. J. Wang: None. R. Wu: None. S. Krishnan-Sarin: None. R. Makuch: None. J. Staley: None. S. O'Malley: Part 1; ACNP Work Group, Alcohol Clinical Trial Initiative, sponsored by Eli Lilly, Janssen, Schering Plough, Lundbeck, Glaxo-Smith Kline and Alkermes, Advisory Board Gilead Sciences.

114. Increased Monoamine Oxidase A Binding in Prefrontal and Anterior Cingulate Cortex During Acute Cigarette Withdrawal

Jeff Meyer*, Ingrid Bacher, Sylvain Houle, Cynthia Xu, Laurie Zawertailo, Alan Wilson, Alex Soliman, Peter Selby, Tony George, Julia Sacher, Laura Miler, Steve Kish, Pablo Rusjan

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

Background: Greater prefrontal and anterior cingulate cortex monoamine oxidase A (MAO-A) binding is associated with sad mood. Substances in cigarette smoke such as harman inhibit MAO-A and cigarette withdrawal is associated with sadness. Dysphoria during cigarette withdrawal predicts relapse. It is unknown whether MAO-A binding rises during early cigarette withdrawal. The objectives of this study are to determine whether prefrontal and anterior cingulate cortex MAO-A binding rises during acute cigarette withdrawal, to determine whether such an effect is related to smoking severity and/or plasma levels of harman, and whether the rise in MAO-A binding is associated with greater sadness.

Method: 24 healthy and 24 otherwise healthy cigarette smoking subjects underwent [^{11}C] harmine positron emission tomography scanning at a tertiary care psychiatric hospital. Healthy subjects were scanned once. Cigarette smoking subjects were scanned twice: after acute withdrawal and after cigarette smoking. MAO-A V_T , an index of MAO-A density, was measured in prefrontal and anterior cingulate cortex.

Results: During withdrawal, prefrontal and anterior cingulate cortex MAO-A V_T rose significantly in the heavy smoking group (22% and 31%; $F_{1,22} = 20.9$ and 28.1 , $p < 0.001$ and $p < 0.001$ respectively) and was greater than healthy subjects ($F_{1,34} = 23.46$ and 24.88 , $p = 0.001$ and 0.001). In the heavy smoking group, the rise in MAO-A V_T in these regions correlated significantly with change in plasma harman ($r = 0.61$ and 0.58 , $p = 0.001$ and $p = 0.001$). The rise in MAO-A V_T was significantly correlated with greater sadness ($r = 0.73$ and $r = 0.70$, $p < 0.001$ in prefrontal and anterior cingulate cortex respectively).

Discussion: The rise in prefrontal and anterior cingulate cortex MAO-A binding associated with reductions in harman during withdrawal from heavy cigarette smoking represent a new, additional, mechanism to explain why sad mood occurs during withdrawal from heavy cigarette smoking. These results argue that the association between cigarette smoking and suicide may relate to quitting heavy cigarette smoking. They also argue for additional clinical trials of MAO-A inhibitors for quitting heavy cigarette smoking.

Disclosure: J. Meyer: Part 1; Lundbeck, Bristol-Myers Squibb, SK Life Sciences. Part 4; GlaxoSmithKline. I. Bacher: None. S. Houle: Part 1; Bristol-Myers Squibb. Part 4; GlaxoSmithKline. C. Xu: None. L. Zawertailo: None. A. Wilson: None. A. Soliman: None. P. Selby: Part 1; Schering, Johnson and Johnson, Pfizer, GlaxoSmithKline, Sanofi-Synthelabo, NABI Pharmaceuticals, Genpharm and Prempharm. Part 4; V-CC Systems, eHealth Behavior Change. T. George: None. J. Sacher: None. L. Miler: None. S. Kish: None. P. Rusjan: None.

115. Increased Ratio of Apoptosis Marker Caspase 8 to a Proliferation Marker in the Orbitofrontal Cortex in Alcoholism, Major Depression, and Comorbid Depression and Alcoholism

Jose Javier Miguel-Hidalgo*, Angela Whittom, Madhav Soni, Grazyna Rajkowska, Ashish Meshram, Craig Stockmeier

University of Mississippi Medical Center, Jackson, MS

Background: Low densities of glial cells in the prefrontal cortex in alcoholism and depression, and age-dependent decreases of neuronal density in the orbitofrontal cortex (ORB) in alcoholism suggest that altered glial cell turnover or glial cell death may jeopardize the survival of specific neural cell types in these psychiatric disorders. We examined the possibility that caspase 8 (FLICE, MACH), a protease involved in initiating apoptosis, and its active form are increased, and that the ratio of caspase 8 to PCNA (a marker for cells undergoing proliferation) is also higher in the

ORB in major depressive disorder (MDD), alcoholism and comorbid depression/alcoholism as compared to non-psychiatric control subjects.

Methods: Punches from the postmortem ORB gray matter were homogenized and processed for detection of caspase 8 or PCNA levels in non-psychiatric control subjects (CONT), and subjects diagnosed with alcohol-dependence (ALC), MDD, or comorbid MDD plus alcohol dependence (MDA). Antibodies against caspase 8 (including pro-caspase 8, intermediate forms of caspase 8 and active caspase 8), and against PCNA were used to quantify those proteins in Western blots. **Results:** Active caspase 8 and some of its intermediate forms were significantly increased in MDD, MDA and ALC as compared to control subjects. The highest levels of caspase 8 were observed in subjects with comorbid MDD and alcoholism (MDA). The highest levels of PCNA were observed in controls subjects, and there was a significantly higher ratio of the apoptotic marker active caspase 8 to PCNA in all three diseased groups as compared to control subjects. In histological sections of the postmortem ORB, cells immunopositive for caspase 8 or PCNA were mostly of small size, possibly glial cells.

Discussion: The present results suggest that an increase in the relative activation of caspase 8 against PCNA is a shared feature in the ORB in depression and alcoholism and may partially explain the pathological vulnerability of some cell types observed in previous studies.

Disclosure: J. Miguel-Hidalgo: None. A. Whittom: None. M. Soni: None. G. Rajkowska: None. A. Meshram: None. C. Stockmeier: None.

116. Reduced Brain Dopamine Related Reward Sensitivity in Obesity

Guido Frank*, Megan Shott, Michael Rollin

University of Colorado Denver, Aurora, CO

Background: The brain mechanisms that contribute to overeating and obesity (OB) are poorly understood. We recently reported that underweight individuals with anorexia nervosa have increased dopamine (DA) related neuronal response to unexpected taste reward delivery or omission (Temporal Difference Model, TD, O'Doherty et al 2003), suggesting a DA hyper-sensitivity. In this study we wanted to test whether individuals with OB have reduced response to unexpected delivery or omission of taste rewards.

Methods: Twenty-one OB (mean age 30 ± 7 years) and 23 healthy control women (CW, mean age 27 ± 5 years) were recruited. Subjects underwent functional magnet resonance brain imaging (fMRI) while receiving three types of solutions (1 mL per trial): 1 M Sucrose, Artificial Saliva (25 mM KCl, 2 mM NaHCO₃) or No-Solution; prior to each solution delivery subjects saw a fractal that predicted the solution type to be delivered. The first ten trials were learning trials for Sucrose-fractal association (discarded from the analysis); the remaining 270 trials were fully randomized; Sucrose and No-Solution were applied 100 times each, and Artificial Saliva was delivered 80 times. Twenty percent of the Sucrose deliveries were preceded by the No-Solution fractal (Unexpected Sucrose), and 20% of the No-Solution deliveries were preceded by the Sucrose fractal (Unexpected No-Solution). A 3T GE scanner acquired T2* weighted echo-planar imaging (EPI) scans to measure blood oxygen-level dependent (BOLD) functional activity during taste stimulation (2.6 x 2.6 x 2.6 mm voxels, TR = 2 seconds, TE = 30 ms, flip angle of 90 degrees, 30 slices, with 2.6 mm slice thickness and 1.4 mm gap). Images were analyzed using SPM5 (FIL London, UK). The following contrast images were created within group and compared across groups in a 2nd level analysis: 1. Receiving Sucrose Unexpectedly (Expect NO + Receive SU)-(Expect NO + Receive NO), 2. Receiving No Solution Unexpectedly (Expect SU + Receive NO) -(Expect SU + Receive SU).

Results: OB subjects were at a higher body mass index (BMI, 34.3 ± 5) compared to CW (21.3 ± 1 ; $p < 0.001$). For the Receiving Sucrose Unexpectedly condition (threshold $p < 0.005$, 75 voxels contiguity), OB versus CW individuals showed significantly reduced clusters ($p < 0.001$, corrected) of activation in the bilateral insula/frontal operculum (MNI coordinates $x = 34, y = 32, z = -4$; $x = -30, y = 26, z = -8$), bilateral ventral putamen (MNI coordinates $x = 22, y = 16, z = -2$; $x = -20, y = 6, z = -2$) extending into the bilateral amygdala (MNI coordinates $x = 26, y = -2, z = -10$; $x = -30, y = 26, z = -8$). For the Receiving No Solution

Unexpectedly (threshold $p < 0.005$, 75 voxel contiguity), OB versus CW individuals showed significantly reduced clusters ($p < 0.001$, corrected) of activation in the bilateral putamen (MNI coordinates $x = 26, y = 14, z = -8$; $x = -18, y = 8, z = -4$), bilateral insula/frontal operculum (MNI coordinates $x = 34, y = 28, z = -10$; $x = -28, y = 26, z = -6$), as well as superior (MNI coordinates $x = 4, y = 48, z = 32$) and inferior (MNI coordinates $x = 4, y = 40, z = -6$) medial prefrontal cortex.

Discussion: This is the first study that explores taste reward function in OB using the TD model. The results indicate that OB individuals have reduced activation in the dopaminergic putamen, but also insula/frontal operculum, amygdala and medial prefrontal cortex, compared to CW. The insula as the primary taste cortex has direct connections to the dopaminergic basal ganglia, and these results propose that OB individuals under-respond to taste stimuli in the primary taste cortex, and this might be related to reduced DA activation. We recently found increased DA related activation in anorexia nervosa individuals using the same paradigm, and these findings then may suggest an adaption of the DA system in humans to the amount of food eaten. That is, OB individuals might desensitize to food stimuli which could lead to inadequate negative feedback and satiety. OB is not only a general major health concern, but also a common side effect of psychiatric medications, and these results help identify anatomical and neurotransmitter related disturbances that could be targeted for intervention in the future.

Disclosure: G. Frank: None. M. Shott: None. M. Rollin: None.

117. Intensity of Smoking Cue-Triggered Brain Activity is Driven by Genetic Variance in DAT1 and COMT

Teresa Franklin*, Ze Wang, Yin Li, Falk Lohoff, Jesse Suh, Marina Goldman, Rebecca Hazan, Daniel Willard, Joshua Shin, John A. Detre, Charles O'Brien, Anna Rose Childress

University of Pennsylvania School of Medicine, Philadelphia, PA

Background: Using arterial spin labeled (ASL) perfusion MRI as a biomarker of brain activity, we previously demonstrated and confirmed a role for dopamine transporter (DAT1) genotype in modulation of smoking cue (SC) reactivity. Given that catechol-o-methyltransferase (COMT) genotype also affects dopaminergic transmission we hypothesized a gene-gene epistatic interaction modulation of brain and behavioral responses to SCs. The DAT is abundantly present in the ventral striatum (VS) and rapidly clears DA after its phasic release in response to rewards and reward predictors. The DAT1 has two common alleles with either a 9- or 10-variable number tandem repeat (VNTR) of a 40 base pair sequence in its 3' untranslated region. The 9-repeat allele has been associated with lower DAT expression. Lower DAT expression may lead to slower DA reuptake such that it lingers longer in the synapse during smoking, prolonging the reward message and strengthening the associations between nicotine and SCs. As the incentive value of SCs is enhanced, so is the craving and associated brain activity triggered in their presence. Indeed, in Franklin et al 09, NPP and again in Franklin et al In Press, Addiction Bio we observed greater responses to SCs in the interconnected medial orbitofrontal cortex (mOFC) and VS in 9-repeat carriers compared to 10/10-repeat homozygotes (homozygotes for the 9-repeat are rare and thus were not examined separately). COMT metabolizes DA and the other monoamines following their phasic release in response to rewards and reward signals. As the DAT is virtually absent at cortical synapses, DA regulation in the prefrontal cortex (PFC) is tightly regulated by COMT catabolism. COMT carries a val158met functional polymorphism wherein the val allele is associated with more efficient enzymatic activity, resulting in less available synaptic DA. DA plays an inhibitory role in the PFC, thus less synaptic DA in the PFC results in less inhibition and thus greater excitability of downstream limbic regions in response to rewards and reward-related stimuli. As PFC excitatory afferents synapse on the terminals of the ventral tegmental area dopaminergic neurons that project to the VS, DA levels may be

enhanced and as suggested above, increased DA may lead to greater brain activity and craving in the presence of SCs. We hypothesized that ventral striatal activity would be greater in smokers who carried a 9-repeat allele, as well as, in those who carried a val allele and further, that there would be an interaction between these genotypes such that activity would be greatest in smokers carrying both a 9-repeat and a val allele.

Methods: Using ASL perfusion fMRI we imaged smokers ($N = 43$, data acquisition is ongoing) who were genotyped for the DAT and COMT polymorphisms during SC and nonSC 10-minute audio-video clips. The SC video featured individuals of various race, age and sex who smoke and use explicit language designed to induce appetitive desire for a cigarette (e.g. "I love a cigarette after a hard day at work."). The nonSC video was similar in content, except individuals related short stories that did not portray cigarette smoking or smoking reminders. Individuals carrying at least one copy of the 9-repeat allele were grouped as 9-repeats and homozygotes for the 10-repeat allele were grouped as 10/10-repeats. Individuals carrying at least one copy of the met allele were grouped as val/mets and those homozygous for the val allele were grouped as val/vals. Sample size precluded the examination of homozygotes for the met allele.

Results: A main effect of DAT genotype was observed in the medial OFC/VS during exposure to SCs (versus nonSCs) with 9-repeats exhibiting greater activity than 10/10-repeats. There was also a main effect of COMT genotype in the VS, mOFC and insula with val/vals exhibiting the greatest activity in VS and mOFC compared to val/mets. An interaction was observed such that the 9-repeat/val/val probands exhibited the greatest responses bilaterally in the VS, amygdala, thalamus and dorsolateral PFC.

Discussion: Taken together, these results indicate genetic variation with functional impact on DA transmission related to SC exposure and may identify a SC-reactive endophenotype. These results support a growing literature demonstrating a role for the DAT 40 base pair sequence and the COMT val158met polymorphisms in brain responses during tasks of impulsivity, reward (anticipation, delivery), and cognition (attention, working memory, executive function). Additionally, variance in DAT and COMT has been shown to be involved in disorders often comorbid with addiction such as schizophrenia, bipolar disorder, depression and ADHD.

Disclosure: T. Franklin: None. Z. Wang: None. Y. Li: None. F. Lohoff: None. J. Suh: None. M. Goldman: None. R. Hazan: None. D. Willard: None. J. Shin: None. J. Detre: Part 4; Wyeth, Astra Zenica. C. O'Brien: Part 1; Reckitt-Benkizer, Embera, Gilead, Catalyst, Alkermes, Abbott. A. Childress: Part 1; Abbott. Part 2; University of Pennsylvania School of Medicine.

118. A Comparison of Longitudinal Volumetric Brain Changes Between Patients With First Episode And Chronic Schizophrenia

Joseph Friedman*, David Carpenter, Cheuk Tang, Vladan Novakovic, Jared Cooper, Marina Goldenberg, Stephanie Gottlieb, Emily Eaves, Johnny Ng, Kenneth Davis

Mount Sinai School of Medicine, New York, NY

Background: Longitudinal magnetic resonance imaging (MRI) studies in first-episode and chronic schizophrenia have demonstrated volume reduction in cortex. However, there are only few longitudinal studies that directly compare progressive brain changes in first-episode and chronic schizophrenia. Therefore we conducted a longitudinal MRI study to investigate potential differences in volumetric brain changes at various stages of the schizophrenia illness (first episode and chronic).

Methods: The following four groups of subjects received baseline and follow-up (minimum 3 years) MRI scans: first episode schizophrenic patients and their individually age and gender matched healthy comparisons, and chronic schizophrenic patients and their individually age and gender matched healthy comparisons. All imaging was performed on a 3T Allegra MRI scanner (Siemens,

Ehrlangen, Germany). The following structural scans were acquired: Axial 3D-MPRage (TR = 2500 ms, TE = 4.4 ms, FOV = 21 cm, matrix size = 256x256, 208 slices with thickness = 0.82 mm); Turbo spin echo (TSE) T2-weighted Axial (TR = 5380 ms, TE = 99 ms, FOV = 18.3x21 cm, matrix = 512x448, Turbo factor = 11, 28 slices, thickness = 3 mm skip 1 mm). Images were processed using the FSL 4.1.5 from Oxford University (<http://www.fmrib.ox.ac.uk/fsl/>). Warp fields were calculated to transform images between Montreal Neurological Institute (MNI) standardized space and each individual's native scan space. The anatomical regions of interest were defined in MNI space using the atlases provided with FSL (the Harvard-Oxford cortical and subcortical atlas as well as the JHU-ICBM white matter atlas). ROIs were then transformed to each subject's native space for individual volumetric quantification on the high resolution T1 image. Rate of volumetric change was calculated by dividing the change in volume by the follow-up period (in years). ANCOVA was conducted using baseline volume and whole brain volume change as co-variates.

Results: To date, 14 first episode schizophrenic subjects (mean age = 26.67, mean follow-up = 3.77) and 14 matched controls (mean age = 27.19, mean follow-up = 3.78), along with 15 chronic schizophrenic subjects (mean age = 43.68, mean follow-up = 4.31) and 14 matched controls (mean age = 43.56, mean follow-up = 4.24) have received baseline and follow-up scans. First episode schizophrenic subjects demonstrated a 1.47%/year volume increase in anterior cingulate ($p = .05$), whereas chronic schizophrenic subjects showed non-significant 0.68%/year decrease ($p = .12$).

Discussion: These preliminary results suggest that the trajectory of brain structural changes in schizophrenia are not homogenous throughout different stages of the illness.

Disclosure: J. Friedman: None. D. Carpenter: None. C. Tang: None. V. Novakovic: None. J. Cooper: None. M. Goldenberg: None. S. Gottlieb: None. E. Eaves: None. J. Ng: None. K. Davis: Part 1; Janssen, Shire.

119. Alterations of Anterior Cingulate Cortex Gamma-Aminobutyric Acid in Adolescent Depression

Vilma Gabbay*, Rachel G. Klein, Xiangling Mao, Aviva Panzer, Benjamin Ely, Daisy Gonzalez, Dikoma Shungu

New York University School of Medicine, New York, NY

Background: Adolescent major depressive disorder (MDD) is a serious public health concern, associated with significant morbidity and suicide risk. To date, only a limited number of neurobiological studies have addressed pediatric MDD populations, which are essential for understanding MDD etiology as these subjects are relatively free from the confounding effects of treatment, chronicity, and aging, promoting the capture of neurobiological processes early in the course of illness. Over the past decade, increasing evidence has implicated the major inhibitory amino acid neurotransmitter gamma-aminobutyric acid (GABA) in the pathophysiology of MDD. Advances in proton magnetic resonance spectroscopy (^1H MRS) spectral editing techniques now allow *in vivo* assessment of uncontaminated GABA concentrations in the brain. Using these techniques, decreased GABA concentrations were reported in the occipital cortex and anterior cingulate cortex (ACC) of adults with MDD. In this study, we sought to extend these findings to adolescent MDD, focusing on the ACC, which has been strongly implicated in the pathophysiology of the disorder, to test the hypotheses that: (i) adolescents with MDD have decreased ACC GABA concentrations relative to healthy comparison subjects, and; (ii) there is a negative association in adolescents with MDD between ACC GABA concentrations and MDD severity, as assessed by the Children's Depression Rating Scale-Revised (CDRS-R).

Methods: Subjects: Seventeen subjects with adolescent-onset MDD (ages 12-19) and 22 healthy controls (ages 13-19) were enrolled. Subjects were evaluated by a child and adolescent psychiatrist

or psychologist using the Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version. MDD subjects met DSM-IV-TR criteria, had episode durations of at least 8 weeks, CDRS-R severity scores of 38 or greater, and were psychotropic medication-free for at least 3 months prior to scan. Healthy controls were psychotropic medication-naïve and did not meet criteria for any DSM-IV-TR diagnosis. All subjects had negative day-of-scan urine toxicology tests, including benzodiazepines. **Data Acquisition and Analysis:** Clean GABA signal detection was achieved by ¹H MRS on a GE 3.0 T "EXCITE" MR system and an 8-channel phased-array head coil using the standard J-editing difference method. GABA levels were expressed semi-quantitatively as ratios relative to the unsuppressed ACC voxel tissue water (w), GABA/w, which will henceforth be referred to simply as GABA. An unequal variance *t*-test was used to compare groups in terms of GABA levels. Pearson correlation coefficients assessed the association of GABA with CDRS-R severity scores. All reported *p* values are two-tailed and are considered statistically significant when less than 0.05.

Results: Mean ACC GABA levels in adolescents with MDD were significantly decreased compared to those in controls (0.0023 ± 0.0004 vs. 0.0027 ± 0.0003 , $t = 2.96$, $df = 25$, $p = 0.007$). Additionally, we found a negative correlation between CDRS-R scores (52.1 ± 8.0) and ACC GABA levels in the MDD group ($r = -0.61$, $p = 0.01$). Furthermore, subjects with melancholic MDD, the most severe subtype, had GABA levels within the lower end of the range.

Discussion: Our findings support a role for GABA alterations early in MDD onset. Interestingly, this is the only study to identify relationships between cortical GABA levels and MDD severity, emphasizing the need for neurobiological research in pediatric populations. Further, our group's recent findings of GABA abnormalities in treatment-resistant depression highlight the need for additional research in MDD subtypes.

Disclosure: V. Gabbay: None. R. Klein: None. X. Mao: None. A. Panzer: None. B. Ely: None. D. Gonzalez: None. D. Shungu: None.

120. In Vivo Binding Of Antipsychotics To D₃ And D₂ Receptors:

A PET Study In Baboons With [11C]-(+)-PHNO

Ragy Gergis*, Xiaoyan Xu, Nobumi Miyake, Balu Eswaramoorthy, Roger Gunn, Eugenii Rabiner, Anissa Abi-Dargham, Mark Slifstein

Columbia University and New York State Psychiatric Institute, New York, NY

Background: Measuring the *in vivo* occupancy of antipsychotic drugs at dopamine D₂ and D₃ receptors separately has been difficult due to lack of selectivity of available tracers. The recently developed [11C]-(+)-PHNO is D₃-preferring, allowing estimates of the relative D₂ and D₃ binding of antipsychotic drugs. We used PET imaging in baboons with [11C]-(+)-PHNO to examine the binding to D₂ and D₃ receptors by clozapine (CLOZ) and haloperidol (HAL).

Methods: Four animals (A,B,C,D) were scanned (all scans 120 minutes and data were analyzed by 2-tissue compartment modeling with arterial input). First, test and retest scans were acquired in single scanning sessions for three subjects (A,B,C) to assess the reproducibility of [11C]-(+)-PHNO scans. The observed percent change in BPND (Δ BPND) following the second scan was less than 10% in putamen (PUT) and caudate (CAD), whereas there were decreases in excess of 20% in all D₃-rich regions, suggesting a mass carryover effect due to the high affinity of [11C]-(+)-PHNO for D₃ (Table 1). Subsequent studies included only one scan per session. Post-antipsychotic drug only-scans were acquired in three subjects (B,C,D, CLOZ 0.5534 mg/kg, HAL 0.0109 mg/kg, twice for each drug in each subject) and compared to baselines, which were the average of 2 (D) or 3 (B,C) scans per subject. HAL and CLOZ were each administered as a slow bolus 15 minutes prior to the scan. Δ BPND following challenge with antipsychotic drugs was measured.

A regression model based on literature values of regional D₂ and D₃ fractions of [11C]-(+)-PHNO BPND in PUT, CAD, substantia nigra (SN), globus pallidus (GP), ventral striatum (VST), and thalamus (THA) was used to infer occupancy at D₂ and D₃ receptors, according to the equation $\text{BPND}(\text{drug condition}) = \text{BPND}(\text{Baseline}) * (\text{fD}_3 * (1-\text{occ}(\text{D}_3)) + \text{fD}_2 * (1-\text{occ}(\text{D}_2)))$ where fD₃ and fD₂ are the fractions of [11C]-(+)-PHNO BPND attributable to each receptor type, occ is the drug occupancy at each receptor type, and ED₅₀(D₃)/ED₅₀(D₂) was computed as $[\text{occ}(\text{D}_2)/(1-\text{occ}(\text{D}_2))] / [\text{occ}(\text{D}_3)/(1-\text{occ}(\text{D}_3))]$.

Results: Δ BPND following antipsychotic challenge is reported in Table 2. The regression model estimated D₂:D₃ selectivity as 5.25 for CLOZ and 2.38 for HAL.

Discussion: The selectivity was very similar to published *in vitro* values for HAL (3.03) but slightly larger for CLOZ (2.82). These data suggest that acute doses of CLOZ and HAL bind to D₃ receptors *in vivo*, and that the lack of D₃ occupancy by antipsychotics observed in some recent imaging studies may be due to other phenomena.

Table 1. $-\Delta$ BPND (%) for Test/Retest studies using [11C]-(+)-PHNO (Mean [SD]). N=3 for all results except GP where n=2 due to nonconvergence of kinetic analysis for one study. PUT: $-\Delta$ BPND = 1% [12%]; p = 0.91 CAD: $-\Delta$ BPND = 7% [11%]; p = 0.34 VST: $-\Delta$ BPND = 22% [9%]; p = 0.04 GP: $-\Delta$ BPND = 42% [18%]; p = 0.33 SN: $-\Delta$ BPND = 29% [32%]; p = 0.23 THA: $-\Delta$ BPND = 29% [16%]; p = 0.11 Table 2. $-\Delta$ BPND (%) (Mean [SD]) for acute-dose antipsychotic study. N = 6 for all results (3 subjects * 2 post-drug studies for each drug). PUT: CLOZ $-\Delta$ BPND = 43% [11%]; HAL $-\Delta$ BPND = 70% [14%] CAD: CLOZ $-\Delta$ BPND = 44% [10%]; HAL $-\Delta$ BPND = 69% [13%] VST: CLOZ $-\Delta$ BPND = 24% [17%]; HAL $-\Delta$ BPND = 66% [18%] GP: CLOZ $-\Delta$ BPND = 21% [14%]; HAL $-\Delta$ BPND = 61% [14%] SN: CLOZ $-\Delta$ BPND = 7% [12%]; HAL $-\Delta$ BPND = 39% [50%] THA: CLOZ $-\Delta$ BPND = 14% [10%]; HAL $-\Delta$ BPND = 31% [75%].

Disclosure: R. Gergis: Part 1; Janssen, Lilly, Forest, Elsevier Science. Part 2; Lilly. X. Xu: None. N. Miyake: None. B. Eswaramoorthy: None. R. Gunn: Part 5; GSK. E. Rabiner: GSK. A. Abi-Dargham: Part 1; BMS-Otsuka, Boehringer-Ingelheim, Lundbeck, Sanofi-Aventis. Part 4; GSK. M. Slifstein: Part 1; Amgen, GSK. Part 4; Intracellular Therapies.

121. The Effect of Family History of Panic on Cortical GABA Deficits in Panic Disorder: A Prospective ¹H-MRS Study.

Andrew Goddard*, Ulrike Dyak, Zaiyang Long, Carla Medlock, Anantha Shekhar, Chris McDougle

Indiana University School of Medicine, Indianapolis, IN

Background: Deficits of GABA neuronal functioning have been implicated in the pathophysiology of PD by recent proton magnetic resonance spectroscopy (¹H-MRS)(Goddard, Mason et al. 2001; Ham, Sung et al. 2007) and by GABA_A-benzodiazepine receptor PET studies (Malizia, Cunningham et al. 1998; Hasler, Nugent et al. 2008) (Cameron, Huang et al. 2007). The hypothesis of the present pilot study is that low cortical GABA levels in PD are familial and are due to dysfunctional polymorphisms of genes involved in GABA neurotransmission, such as those that code for the GABA synthetic enzymes GAD₆₅ or GAD₆₇, glutamine synthetase (GS), or for the GABA transporter, GAT-1. Based on preliminary family history/cortical GABA data (Goddard, Mason et al. 2004b), we expect that cortical GABA deficits in family history positive PD patients will be more profound vs those observed in family history negative patients, due to allelic variation in genes that encode for enzymes and proteins involved in GABA metabolism and neurotransmission.

Methods: We are therefore conducting a preliminary, prospective, parallel-group comparison study of ¹H-MRS-cortical GABA levels obtained from 2 brain ROIs (anterior cingulate cortex (ACC) and occipital cortex (OCC)) in 3 mutually-exclusive human subject groups: 1) PD patients with a positive PD family history (1st degree relatives), 2) without a family history of PD, and 3) age- and sex-matched healthy control subjects (HCs) without a family history of psychopathology. DSM-IV PD diagnoses are established by SCID interview, and family

history status with the Family Health Inventory (FHI) (Merikangas 1993). Subjects are free of psychotropic medicines for 4 weeks prior to imaging. 10 cc of whole blood is collected from each consenting subject into a plastic tube and stored in a -80°C freezer for future candidate gene/DNA analysis. Short echo time (TE) spectra (TE = 30 ms), and GABA-edited spectra (TE = 68), are obtained from each ROI. The short TE spectra will provide an estimation of water, creatine, glutamate, glutamine, NAA, aspartate and choline. The GABA peak is detected by application of the homonuclear MEGA-PRESS J-editing sequence (Mescher, Merkle et al. 1998), adapted for the detection of GABA (Eden and Barker 2007). Concentration ratios of NAA, Creatine, choline (Cho), myo-inositol (mI), lactate (lac), glutamine + Glu (Glx), Glu, and GABA to brain water are obtained by fitting the raw spectroscopy data with a linear combination model of basis spectra (LCModel)(Provencher 1993).

Results: We have collected ACC ROI data in n=7 PD patients (4 females and 3 males) and n=7 healthy comparison subjects, and, based on these observations, estimated (assuming an NAA concentration = 11 mM) the following mean \pm SD cortical GABA values for this ROI: family history positive PD patients (n=3) were $= 0.49 \pm 0.51$ mmol/L; family history negative PD patients (n=4) were $= 2.3 \pm 1.0$ mmol/L; and volunteer subjects (n=7) were $= 1.7 \pm 0.58$ (mmol/L). OCC ROI data are currently being analyzed.

Discussion: Thus far, our pilot data are supportive of the main study hypothesis, in that family history positive PD patients have low ACC GABA levels vs the other comparison groups. If confirmed with additional work, this clinical subtype of PD may be significant in terms of having a unique pathophysiology and selective response to GABAergic treatments.

Disclosure: A. Goddard: Part 1; BMS-consultant; Orexigen-consultant; Janssen-grant awardee; Astra-Zeneca-grant awardee; Pfizer-consultant. Part 2; Orexigen-consultant. Part 4; Janssen-independent grant; Astra-independent grant. U. Dydak: None. Z. Long: None. C. Medlock: None. A. Shekhar: None. C. McDougle: None.

122. An *In Vivo* fMRI Human Study of Fetal Programming of the Stress Response in the Brain: Implications for Depression

Jill Goldstein*, Anne Klibanski, Stephen Buka, Brandon Abbs, Laura Holsen, Nikos Makris, Mady Hornig

Brigham & Women's Hospital; Harvard Medical School, Boston, MA

Background: Fetal risk factors for depression have been identified in human population-level studies. However, at the level of the brain, this has been primarily studied in animals, in particular, modeling prenatal stress. These models have implicated disruption of the hypothalamic-pituitary-adrenal (HPA) circuitry (or stress response circuitry) and inflammatory factors as key to understanding risk for major depressive disorder (MDD). Here we present a unique *in vivo* functional magnetic resonance imaging (fMRI) study of *fetal* programming of the stress response in the adult brain. We have the rare opportunity to investigate this *in vivo* given that we have followed and brain imaged a prenatal cohort at ages 35-48 who have prenatal maternal sera stored 40-50 years ago.

Methods: In a 50-year prenatal cohort study (from 1959-2010), for whom mothers were followed through their pregnancies and sera stored at NIH, we identified in the adult offspring 500 cases of MDD comparable to 700 healthy controls. Fetal inflammatory conditions (e.g., fetal growth restriction (FGR) and preeclampsia) were assessed and pro-inflammatory cytokine assays were conducted in maternal prenatal sera at mid-to-late gestation (cytokines associated with the HPA: IL-1 β , IL-6, IL-8, IL-10, TNF- α). A subsample of subjects was scanned as adults using fMRI in studies of the stress response circuitry. fMRI data will be presented for 30 healthy subjects (half of whom were exposed to FGR and half unexposed) compared with 15 additional subjects with recurrent DSM-IV MDD in remission made comparable to healthy controls

on sociodemographics and right-handedness. Subjects viewed images with negative valence/high arousal versus neutral valence/low arousal stimuli. A 3T Siemens MR scanner was used and SPM8 for imaging analyses of blood oxygen-level dependent (BOLD) signal changes in hippocampus (HIPP), amygdala, anterior hypothalamus (aHYPO), orbitofrontal cortex (OFC), medial prefrontal cortex (mPFC), and anterior cingulate gyrus. Baseline fasting and hormonal assays collected during scanning (timed to pituitary and steroid hormone responses) included adrenal (DHEAS, cortisol) and gonadal (estradiol, progesterone, testosterone) hormones. General linear models were used to relate adult hormone and prenatal cytokine levels to BOLD changes in healthy controls with and without FGR compared with MDD cases. Clinical state was also assessed pre- and post-scanning.

Results: We demonstrated significant BOLD changes in stress response circuitry dependent on exposure to FGR and MDD status by sex. MDD women and FGR-exposed healthy women demonstrated BOLD *hyperactivity* compared to non-FGR exposed healthy women in HIPP, OFC and mPFC, and *hypoactivity* in the aHYPO, with effect sizes of .3-.75 standard deviation differences in BOLD signal. MDD women had low estradiol and high progesterone compared with healthy women, which had opposing significant effects on the aHYPO ($\beta = .79$ E2; $\beta = -.89$ PRG) and HIPP BOLD signal. Higher HIPP activity was also significantly associated with higher levels of DHEAS in response to stressful stimuli ($r = .62$). In addition, hyperactivity in HIPP and OFC were significantly associated with higher prenatal IL-10 and IL-8, which were higher in women with MDD (in GLM, t's at $p < .03$). IL-6 had the greatest effect on hypoactivity of the aHYPO ($t = -4.69$, $p < .02$).

Discussion: Findings suggest that in humans, as in animal studies, fetal inflammatory conditions, operationalized here as FGR and prenatal pro-inflammatory cytokines, have a significant impact on the functioning of the stress response circuitry in adulthood 40-50 years later. Stress response circuitry dysregulation was significantly associated with gonadal and adrenal hormonal dysregulation in adulthood and fetal inflammatory conditions during mid-to-late gestation, which characterized MDD. Thus, in a human *in vivo* model we demonstrate that understanding the *fetal* programming of the stress response circuitry in the brain is important for understanding vulnerability to MDD. Findings have significant implications for the conduct of translational studies given the substantial number of animal studies of prenatal stress that rely on MDD-associated traits as outcomes but cannot study the disorder itself.

Disclosure: J. Goldstein: None. A. Klibanski: None. S. Buka: None. B. Abbs: None. L. Holsen: None. N. Makris: None. M. Hornig: None.

123. Synthesis and MicroPET Imaging of a Fluorinated Nitrogen Heterocyclic Thioflavin Analog as a Candidate β -Amyloid Imaging Agent

Fanxing Zeng, Brian Ciliax, Allan Levey, Mark Goodman*

Emory University, Atlanta, GA

Background: Alzheimer's Disease (AD) is the most common form of dementia. The requisite neuropathologic signature of AD on postmortem brain examination is the deposition of amyloid plaques (A β) and neurofibrillary tangles. Development of small molecular probes for labeling A β plaques in human brain *in vivo* show great promise in identifying and following individuals at risk for AD with high levels of A β , and also in evaluating the efficacy of potential therapeutic interventions. However, development of ligands with improved signal to noise ratio and sensitivity for A β detection would greatly enhance ability to identify patients for therapeutic interventions as early as possible with low levels of amyloid depositions. As part of an ongoing research in our laboratory to develop more sensitive radioligands for A β plaques, a novel fluorinated 5-substituted 2-(2'-fluoro-4'-dimethylaminophenyl)-thiazolo[5,4-b]pyridine

was synthesized and its *in vivo* brain kinetics was studied by microPET imaging in a cynomolgus monkey.

Methods: 2-(2'-Fluoro-4'-dimethylaminophenyl)-thiazolo[5,4-b]-5-fluoropyridine was synthesized from 3-amino-6-fluoro-2-thiopyridine and 4-dimethylamino-2-fluorobenzoic acid by treatment with PPA. 2-(2'-Fluoro-4'-dimethylaminophenyl)-thiazolo[5,4-b]-5-[¹⁸F]fluoropyridine was synthesized from the corresponding 5-chloro precursor with K[¹⁸F] Kryptofix and K₂CO₃ in DMSO in 53% RCY and >95% RCP.

Results: 2-(2'-Fluoro-4'-dimethylaminophenyl)-thiazolo[5,4-b]-5-[¹⁸F]fluoropyridine exhibited good initial brain uptake followed by rapid clearance from cerebellum, frontal and occipital cortex. Peak SUV for cerebellum, frontal and occipital cortex were 2.9, 2.9 and 3.0 at 22.5 min p.i., respectively and 1.9, 1.9 and 1.6, respectively at 120 min.

Discussion: These results suggest that 2-(2'-fluoro-4'-dimethylaminophenyl)-thiazolo[5,4-b]-5-[¹⁸F]fluoropyridine could be a potential agent for detecting human amyloid plaques by PET when labeled with fluorine-18.

Disclosure: F. Zeng: None. B. Ciliax: None. A. Levey: None. M. Goodman: None.

124. Elucidating the Mechanism of Action of Deep Brain Stimulation: an [¹⁸F]Fallypride PET Study in Gilles de la Tourette Syndrome

Gerhard Gründer*, Jens Kuhn, Doris Lenartz, Mardjan Raptis, Wolfgang Huff, Hildegard Janouschek, Irene Neuner, Wolfgang M. Schaefer, Felix Mottaghy, Volker Sturm, Ingo Vernaleken

RWTH Aachen University, Aachen, Germany

Background: Deep brain stimulation (DBS) in Gilles de la Tourette syndrome (GTS) is one of the few accepted surgical treatment options in psychiatry. However, the choice of target areas and stimulation parameters are principally based on empirical knowledge, because the underlying physiological principles of DBS are not entirely understood, yet. In order to elucidate the mechanism of action of DBS, we performed an [¹⁸F]fallypride positron emission tomography study in patients with Gilles de la Tourette syndrome (FP-PET).

Methods: Three patients suffering from treatment resistant GTS (two males, both 22 yrs., one female, 27 yrs.) underwent DBS approximately six months before inclusion into the study. The target regions in two patients were the bilateral mediodorsal nuclei of the thalamus; the third patient received an unilateral thalamic (Ncl. ventrolateralis) stimulation. All three patients improved significantly in their symptomatology. Two PET scans using the high-affinity D_{2/3}-receptor ligand [¹⁸F]fallypride were performed in each patient: The first scan under stimulator-“on”, the second scan under “off”-conditions (electrodes turned off one hour before tracer injection). The scans necessarily had to be performed under anesthesia (propofol/remifentanil) in order to avoid tics during the four hours of acquisition. Time activity curves were obtained after anatomical coregistration and normalization using a standard VOI-template. BPND calculation was performed according to the simplified reference tissue (SRTM) model using the cerebellum as reference region.

Results: Compared to age matched control subjects (without anesthesia) BPND values in the three patients were markedly increased (Putamen: +45 to +52%; Caudate: +49 to +60%; thalamus: +80 to +92%; inf. temporal cortex: +44 to +114%) in the “on”-condition. Turning off the bithalamic stimulation led to a decrease of D_{2/3} receptor availability in the thalamus (7 - 18% decrease of BPND-values). The patient with unilateral left-sided thalamic stimulation responded with a reduced BPND (-6.4%) in the left thalamus, whereas the contralateral right thalamus showed an +16% increase while pausing the DBS.

Discussion: We found spatially selective reductions in D_{2/3} receptor availability between the on- and off-conditions in all three patients (mediodorsal/ventrolateral thalamus). This is most likely due to an increased dopaminergic transmission in the off-condition. Thus, we hypothesize that DBS exerts its beneficial effects in GTS by a profound

reduction in thalamic dopamine release. Especially the lateralized effect in the thalamus of the patient under unilateral stimulation supports this interpretation. The marked increase of binding potentials, however, suggests counter regulatory effects in terms of D_{2/3} receptor up-regulation as a result of chronic DBS. This might be a physiological correlate of the lingering fate of beneficial effects frequently seen in DBS patients.

Disclosure: G. Gründer: Part 1; AstraZeneca, Bristol-Myers Squibb, Johnson & Johnson, Eli Lilly, Wyeth, Servier, Lundbeck, Otsuka, Pfizer, Alkermes. Part 2; Bristol-Myers Squibb, Eli Lilly. Part 3; Bristol-Myers Squibb, Eli Lilly. Part 4; Alkermes, Bristol-Myers Squibb, Eli Lilly, Johnson & Johnson. J. Kuhn: None. D. Lenartz: None. M. Raptis: None. W. Huff: None. H. Janouschek: None. I. Neuner: None. W. Schaefer: None. F. Mottaghy: None. V. Sturm: None. I. Vernaleken: Part 1; Bristol-Myers Squibb, Eli Lilly, Otsuka, Glaxo SmithKline.

125. Dopaminergic Modifications in Treatment-Naïve Patients with OCD Submitted to a Randomized Clinical Trial Between Fluoxetine and Cognitive-Behavior Therapy: A Preliminary Analysis with [^{99m}Tc-TRODAT-1 and SPECT

Marcelo Hoexter*, Roseli Shavitt, Darin Dougherty, Carina D'Alcante, Fabio Duran, Juliana Diniz, Thilo Deckersbach, Suzanne Haber, Geraldo F Busatto, Euripedes Miguel, Rodrigo Bressan

Federal University of Sao Paulo, Clinical Hospital, University of Sao Paulo Medical School, Clinical Hospital, University of Sao Paulo Medical School, Massachusetts General Hospital, Harvard Medical School, Sao Paulo, Brazil

Background: In recent years, molecular brain imaging studies have provided evidence for a possible involvement of the dopaminergic system in the pathophysiology of obsessive-compulsive disorder (OCD). However, to our knowledge, no prior study evaluated changes in the functional anatomy of this neurotransmitter in treatment-naïve adult patients with OCD before and after medication and cognitive-behavior therapy. Thus, we assessed basal ganglia dopamine transporters (DAT) density in treatment-naïve adults with OCD before and after either medication or cognitive-behavior therapy, and in a group of healthy controls. Our prior hypothesizes were that patients with OCD would present basal ganglia DAT density abnormalities compared with controls at baseline and that these abnormalities would be ameliorated after treatment and clinical improvement.

Methods: Forty-one treatment-naïve adults with OCD and 32 healthy controls matched for age, gender, socioeconomic status, level of education and handedness underwent a [^{99m}Tc-TRODAT-1 and single photon emission computerized tomography (SPECT) scan at baseline. Patients were then sequentially allocated to receive either fluoxetine (up to 80 mg/day) or group cognitive-behavior therapy (CBT) (weekly 2-h sessions) for 12 weeks. Post-treatment TRODAT-1 and SPECT scans were also conducted in 28 patients (14 in the fluoxetine group and 14 in the CBT group) who completed the entire protocol after 12 weeks. SPECT images were coregistered with structural magnetic resonance imaging (MRI) and DAT binding potential (DAT-BP) for bilateral caudate, anterior putamen and posterior putamen was calculated using the following formula: specific DAT binding in the region of interest - non-specific DAT binding in the cerebellum / non-specific DAT binding in the cerebellum.

Results: At baseline, OCD patients (n = 41) presented significant differences in DAT-BP compared to healthy controls (n = 32) in the right anterior putamen (mean DAT-BP ± standard deviation: 2.05 ± 0.36 for patients and 2.24 ± 0.37 for controls; p = 0.031) and a statistical tendency in the left anterior putamen (mean DAT-BP ± standard deviation: 2.04 ± 0.40 for patients and 2.27 ± 0.55 for controls; p = 0.071). Patients had lower binding ratios than healthy subjects. There were positive correlations between severity of obsessive-compulsive symptoms (Y-BOCS scores) and DAT availability in left caudate (r = 0.40, p = 0.01), left anterior putamen (r = 0.41, p = 0.008), right caudate (r = 0.37, p = 0.018) and right

anterior putamen ($r = 0.38$, $p = 0.013$). Twenty-eight subjects (14 fluoxetine-treated and 15 CBT-treated patients) completed the full 12-week treatment protocol. As a group, patients exhibited a significant reduction in the OCD symptom severity as measured by the Y-BOCS (reduction of 36%, $p < 0.001$). Individually, the fluoxetine and CBT subgroups displayed significant OCD symptom improvement (reduction of 39%, $p = 0.003$ and 33%, $p = 0.001$ respectively). There were no statistically significant changes in within-group analysis comparing DAT-BP before and after treatment when considering the entire group ($n = 28$) or CBT-treated patients ($n = 14$) in any of the regions investigated. Pre versus post-treatment analysis for fluoxetine-treated patients ($n = 14$) showed a statistical tendency of increment in DAT-BP in left caudate (increase of 11%, $p = 0.064$), left anterior putamen (increase of 11%, $p = 0.084$) and right anterior putamen (increase of 13%, $p = 0.095$).

Discussion: To our knowledge, this is the first study to investigate the functional anatomy of dopaminergic system in a relatively large sample of treatment-naïve adult patients with OCD submitted to a randomized controlled clinical trial between fluoxetine and CBT. Our results showed that OCD patients had lower DAT-BP in regions within the striatum compared to controls, providing evidence for an involvement of the dopaminergic system in the pathophysiology of OCD. Both fluoxetine and CBT were effective in reducing obsessive-compulsive symptoms in treatment-naïve adult patients, but it seems that only fluoxetine treatment was able to modulate dopaminergic function in OCD. Further analyses are necessary to investigate possible neurobiological commonalities and differences between these two modalities of treatment in OCD and to disentangle whether basal ganglia modifications in DAT-BP observed in fluoxetine-treated patients are directly associated with symptoms improvement or are a secondary effect of changes in serotonergic activity.

Disclosure: M. Hoexter: None. R. Shavitt: None. D. Dougherty: None. C. D'Alcante: None. F. Duran: None. J. Diniz: None. T. Deckersbach: None. S. Haber: None. G. Busatto: None. E. Miguel: None. R. Bressan: None.

126. Reduced Activation of the Medial Prefrontal Cortex During Recall of Fear Extinction in Schizophrenia

Daphne Holt*, Garth Coombs, Mohamed Zeidan, Donald Goff, Mohammed Milad

Massachusetts General Hospital, Charlestown, MA

Background: Several lines of evidence suggest that emotional learning and memory mechanisms are disrupted in schizophrenia. First, many of the symptoms of the illness (both positive and negative) are associated with abnormalities in emotion perception and/or learning. Second, schizophrenia has been repeatedly associated with structural and functional abnormalities of the brain circuitry known to support emotional memory, which includes the amygdala, hippocampus and medial prefrontal cortex (mPFC). An experimental approach that has been commonly used in the study of emotional learning in mammals is the Pavlovian fear conditioning paradigm. This paradigm can also be extended, in a two-day procedure, to allow measurement of emotional memory processes, such as fear extinction recall, in humans. In a previous study, we found that fear extinction recall, measured using skin conductance responses (SCR), was reduced in schizophrenia patients in comparison to healthy subjects. In the current study, we sought to identify the changes in brain function associated with this abnormality in schizophrenia, using a two-day fear conditioning and extinction paradigm (similar to the paradigm used in our previous study) and functional MRI.

Methods: Twenty patients with DSM-IV-diagnosed schizophrenia or schizoaffective disorder and 17 healthy control subjects underwent a two-day fear conditioning and extinction procedure, while fMRI data were simultaneously collected. Fear conditioning and extinction learning took place on Day 1 and extinction recall on Day 2 (Milad et al, 2007). During the fear conditioning phase, two distinct visual stimuli were each paired with a mild electrical shock (CS + 1, CS + 2),

while only one of the two was presented during the extinction learning phase (CS + 1), and a third stimulus was never paired with a shock (CS-). Neural responses associated with acquisition of conditioned fear (CS + versus CS-) and those associated with recall of the extinction memory (CS + 1 versus CS + 2) were measured in three regions-of-interest: the amygdala, hippocampus and mPFC. The data were analyzed using the FreeSurfer fMRI analysis stream using a random effects analysis.

Results: There were no differences between the two groups in the acquisition or initial extinction of conditioned fear responses on Day 1, as indexed by SCRs. In contrast, the fMRI analyses revealed that the schizophrenia patients showed reduced amygdala and hippocampal activation, in comparison to the controls, during fear acquisition, which was due to equivalent or greater responses to the CS- relative to the CS + in the schizophrenia group. In a subsample of the cohort with SCR data on both days of the study (13 controls, 11 patients), the schizophrenia patients demonstrated significantly lower extinction recall levels on Day 2 than the controls ($p < .05$). Moreover, in both the full cohort and the subgroup with SCR data, the schizophrenia group exhibited significantly lower responses within the ventromedial PFC than the control subjects ($p < .001$) during extinction recall.

Discussion: In this study, patients with schizophrenia exhibited inappropriate amygdala and hippocampal responses to benign stimuli (the CS-) during fear learning, and failed to recruit the mPFC during delayed fear extinction recall. These findings add to the evidence for an impairment of fear learning and memory mechanisms in schizophrenia and suggest that emotional memory deficits in schizophrenia may arise from dysfunction of the mPFC. Follow-up analyses will determine whether these abnormalities are linked to the symptoms and functional outcomes in the disorder.

Disclosure: D. Holt: None. G. Coombs: None. M. Zeidan: None. D. Goff: Part 1; Over the last two years, Dr. Goff has served as a consultant or advisor to: Xytis, Forest Labs, Pfizer, Indevus Pharmaceuticals, H. Lundbeck, Schering-Plough, Eli Lilly, Takeda, Biovail, Solvay, Hoffmann-La Roche, Janssen, Novartis, and GlaxoSmithKline. M. Milad: None.

127. Functional and Structural Brain Circuits underlying Smoking and Smoking in Schizophrenia

Elliot Hong*, Xiaochu Zhang, Colin Hodgkinson, Yihong Yang, Hemalatha Sampath, Thomas Ross, Brittany Buchholz, Betty Jo Salmeron, Gunvant Thaker, David Goldman, Elliot Stein

Maryland Psychiatric Research Center, Baltimore, MD

Background: Multiple brain regions and circuits have been implicated in smoking using brain imaging. Nicotine addiction is a complex condition that presents with many behavioral correlates implicating both a genetic and brain circuitry foundation. It is disproportionately present in individuals with psychiatric conditions. Most of the previously proposed brain regions, circuits, or mechanisms explain specific aspects of smoking. In a series of recent studies, we found that a circuit involving the dorsal anterior cingulate (dACC) and the ventral striatum/extended amygdala (VS/EA) is associated with several key aspects of nicotine addiction. Using resting state functional connectivity (rsFC) analysis, we initially found that dACC-VS connectivity strength was inversely correlated with nicotine addiction severity (Hong et al 2009; AGP). Subsequently, we found that the nAChR $\alpha 5$ subunit gene functional variant rs16969968 was associated with dACC-VS/EA rsFC, such that (1) the risk allele leads to reduced rsFC in the circuit; (2) this gene-derived rsFC strength was reduced with smokers compared to nonsmokers; and (3) reduction of rsFC in the circuit also predicts more severe nicotine addiction in smokers (Hong et al 2010; PNAS). Using diffusion tensor imaging (DTI), we found multiple brain regions with reduced white matter integrity as measured by fractional anisotropy (FA) in schizophrenia and in smoking independently. The only overlapping FA reduction between the two conditions was localized to the left

anterior thalamic radiation/anterior limb of the internal capsule, a fiber track that connects frontal cortex (including dACC) to the striatum (Zhang et al In Press; *Bio Psychiatry*). In addition, reduced rsFC strength in the same circuit was found in patients with psychiatric conditions that was also independently and additively present along with effect of the nAChR $\alpha 5$ genetic variant (Hong et al 2010). Therefore, these resting state and DTI data provide preliminary converging evidence that this circuit may also be related to the high risk of smoking in psychiatric conditions. New data further suggest that this circuit is related to striatal inhibition functions, with the extent of the striatal inhibition associated with nicotine addiction severity.

Methods: We used gene-circuit analysis, resting fMRI, DTI, event-related fMRI, and circuit-addiction behavior analyses to examine the dACC-VS/EA circuit in smoking.

Results: Our recent effort focuses on the heuristic implication of this circuit impairment by combining event-related fMRI using a GO/NOGO paradigm and resting state fMRI. Preliminary data from 13 smokers and 13 nonsmoker controls were used to test a 2×2 factorial model with a within-subject effect (task: successful and failed inhibition) and a between-subject effect (group: smoker and control). Both striatum and dACC showed significant task activation main effects and dACC showed a significant group main effect. The regression model [dependent variable: activity during successful inhibition in striatum; predictors: all dACC-striatal-subregion rsFC and nicotine addiction severity score] was significant (R^2 -square change = 0.69, $p = 0.001$). The striatal activity during successful go/no-go inhibition was positively associated with addiction severity ($t = 3.2$, $p = 0.005$) and negatively related to the dACC-nucleus accumbens rsFC ($t = -3.1$, $p = 0.006$). No significant relationships were found when using the dACC as the dependent variable. This result suggests that the striatal response during the response inhibition task is significantly associated with the severity of nicotine addiction; and also negatively associated with rsFC strength between dACC-VS, indicating that rsFC may, at least in part, be indexing a response inhibition function such that reduced rsFC in the circuit is related to less controlled striatal activity during inhibitory functioning; the striatal response itself appears linked to nicotine addiction severity.

Discussion: While many brain circuits and mechanisms can explain different aspects of smoking, our multi-modal imaging approach implicates a dACC-ventral striatum/extended amygdala circuit in smoking, and shows that this circuit can tie together aspects of the genetic, behavioral, comorbid, and cognitive correlates of smoking. If validated using confirmatory study designs, a brain circuit capable of incorporating many facets of smoking pathophysiology may provide a particularly salient biomarker for guiding and testing new treatment development.

Disclosure: E. Hong: None. X. Zhang: None. C. Hodgkinson: None. Y. Yang: None. H. Sampath: None. T. Ross: None. B. Buchholz: None. B. Salmeron: None. G. Thaker: None. D. Goldman: None. E. Stein: None.

128. Latent Toxoplasmosis Reduces Gray Matter Density in Schizophrenia but not in Controls. Voxel-Based-Morphometry (VBM) Study.

Jiri Horacek*, Jaroslav Flegr, Jaroslav Tintera, Tomas Novak, Martin Brunovsky, Vera Bubenikova-Valesova, Tomas Palenicek, Cyril Höschl

Prague Psychiatric Centre, Prague, Czech Republic

Background: The role of coccidian protozoa *Toxoplasma gondii* represents one of the most enigmatic and unexplained questions in the field of pathophysiology of schizophrenia. Epidemiological studies suggest that latent toxoplasmosis is associated with a substantially higher risk of schizophrenia but a causal relationship has not been established as yet. To address the role of latent *Toxoplasma gondii*

(*T. gondii*) infection in pathophysiology of schizophrenia we studied the influence of latent toxoplasmosis on brain morphology in schizophrenia.

Methods: Magnetic resonance imaging was analyzed by an optimized voxel-based-morphometry (VBM) in 44 schizophrenic patients (12 *T. gondii* positive) and 56 controls (13 *T. gondii* positive). The full factorial model of analysis of variance with diagnosis and seropositivity for latent toxoplasmosis as factors was used to address the differences in gray and white matter. For all VBM analyses, total brain volume (TBV) was used as a nuisance covariate.

Results: VBM analyses showed the grey matter (GM) volume reduction in schizophrenia patients compared with controls bilaterally in the neocortical regions, hippocampus, middle and posterior cingulate and in the caudate. In the subgroup of patients and controls seropositive to *T. gondii* the reduction of GM was located in the same regions as in the whole sample and consisted of 11660 over-threshold voxels ($p = 0.05$, FWR corrected). The differences between *T. gondii* negative patients and controls consisted only of 289 voxels in temporal and mediotemporal regions. *T. gondii* seropositivity as a factor had no influence on brain volumes in controls but was strongly associated with GM reduction in patients.

Discussion: Our study is the first to document that latent toxoplasmosis reduces GM in schizophrenia but not in controls. The higher morphological vulnerability of patients but not controls to *T. gondii* infection represents an indirect support for the epidemiological evidence of the role of latent toxoplasmosis in schizophrenia. *T. gondii* can affect gray matter by several mechanisms including kynureneine metabolites and dopamine overactivity. Acknowledgments: This work was supported by grants 1M0517 and VZ 0021620816 and 0021620828 from the MSMT CR.

Disclosure: J. Horacek: None. J. Flegr: None. J. Tintera: None. T. Novak: None. M. Brunovsky: None. V. Bubenikova-Valesova: None. T. Palenicek: None. C. Höschl: None.

129. Blood-Oxygenation-Level Dependent Signal Responses to Emotional Stimuli Depend on Variations in rs110402 within the Corticotropin-Releasing Hormone Receptor Gene

David Hsu*, Brian Mickey, Scott Langenecker, Mary Heitzeg, Tiffany Love, Heng Wang, Susan Kennedy, Marta Pecina, Tal Shafir, Colin Hodgkinson, Mary-Anne Enoch, David Goldman, Jon-Kar Zubieta

University of Michigan, Ann Arbor, MI

Background: The corticotropin-releasing hormone (CRH) system coordinates neuroendocrine and behavioral responses to stress, and has been implicated in major depressive disorder (MDD). Several recent reports demonstrate that variations in rs110402, a single nucleotide polymorphism in the CRH receptor 1 (CRHR1) gene, interact with childhood abuse to moderate severity of MDD and responsiveness of the hypothalamic-pituitary-adrenal axis. In particular, the rs110402 GG genotype has been shown to be more vulnerable to stress. The present study uses functional magnetic imaging (fMRI) in healthy controls to examine how variations in rs110402 influence brain responses to emotional stimuli. In addition, genotype frequencies of healthy controls were compared to that of a sample of MDD patients.

Methods: Genomic DNA was extracted from blood and rs110402 was genotyped in 130 healthy controls screened for active medical illness and for psychiatric disorders. 83 subjects completed an fMRI scan while viewing blocks of positive, negative, and neutral words. Carriers of AA/AG genotypes ($n = 50$) were compared with GG-homozygous individuals ($n = 33$). Associations were also sought between personality traits for each of the two genotype groups with levels of brain activity. In addition, genotype frequencies of healthy controls ($n = 130$) were compared to a sample of MDD patients ($n = 20$).

Results: Robust differences in blood-oxygenation-level dependent (BOLD) signal were found for AA/AG > GG while viewing negative words minus neutral words in the right middle temporal/angular gyrus

($t_{81} = 5.61$, $p = 0.01$, FDR-corrected) and in the right frontal pole ($t_{81} = 4.53$, $p = 0.03$, FDR-corrected). These effects were driven by neutral words > negative words in GG-carriers. Greater activity for AA/AG carriers was also found in the bilateral insula, left nucleus accumbens, midline thalamus, and bilateral locus coeruleus. In GG-, but not AA/AG-carriers, middle temporal/angular gyrus activity was positively associated with measures of trait anxiety ($r = 0.49$, $p = 0.01$), neuroticism ($r = 0.42$, $p = 0.03$), vulnerability to stress ($r = 0.40$, $p = 0.04$), and behavioral inhibition ($r = 0.40$, $p = 0.05$); and negatively associated to social well being ($r = -0.49$, $p = 0.01$). GG-carriers trended towards having a higher incidence of MDD ($p = 0.076$).

Discussion: This is the first demonstration that a SNP in the CRHR1 gene is associated with differences in brain activity. Brain activity in carriers of the risk genotype was associated with personality traits related to MDD and anxiety. These results suggest brain mechanisms by which variations of CRHR1 rs110402 respond differently to emotional stimuli, and how this may contribute to differences in emotional responses to stress, and the development of MDD.

Disclosure: D. Hsu: None. B. Mickey: None. S. Langenecker: None. M. Heitzeg: None. T. Love: None. H. Wang: None. S. Kennedy: None. M. Pecina: None. T. Shafir: Part 1; Teva Pharmaceuticals. C. Hodgkinson: None. M. Enoch: None. D. Goldman: None. J. Zubietta: Eli Lilly, Merck. Part 2; Eli Lilly. Part 3; Eli Lilly.

130. Subcortical Dopamine Synthesis is Differentially Associated with Separable Cognitive Factors

Angela Ianni*, Joseph Masdeu, Dwight Dickinson, Philip Kohn, Anees Benferhat, Daniel Eisenberg, Daniel Weinberger, Karen Berman

NIH, Bethesda, MD

Background: The dopamine system is known to modulate cognition¹. In humans, this relationship has been investigated most extensively for working memory, but animal studies document that dopaminergic tone is critical for other cognitive domains as well². For cognitive domains outside working memory, most studies in humans have used indirect indices of dopamine activity, for instance examining neural function in dopamine-rich regions or comparing genetic variants of genes related to dopamine processing, such as DAT or COMT³. Direct measurements of dopamine synthesis have focused on patients with dopamine disorders such as Parkinson's disease⁴. Little is known about how individual variability in dopamine synthesis affects various cognitive processes in healthy adults. To address this open issue, we calculated composite scores based on factor analysis of a comprehensive neuropsychological battery and examined four separable cognitive domains: verbal memory, n-back, processing speed and card-sorting⁵. To search for a dopaminergic neural substrate underlying individual variability in these cognitive domains, we tested the relationship between scores on these factors and subcortical presynaptic dopamine synthesis.

Methods: Forty-nine healthy subjects (mean age 32 ± 10 , range 19-49, 47% women) had neuropsychological testing and, on a separate day, a [¹⁸F]-fluorodopa (FDOPA) positron emission tomography (PET) scan to measure presynaptic dopamine. History, physical examination, and the Structured Clinical Interview for DSM-IV were obtained in order to rule out medical and psychiatric illnesses, substance abuse, and pharmacological treatment. After pretreatment with 200 mg of carbidopa, two 60-second 12 mCi oxygen-15 water rCBF measurements, and injection of 16 mCi of FDOPA, a series of dynamic scans was obtained over 90 minutes. Using SPM5, images were attenuation-corrected, registered, and stereotactically normalized to a standard PET template, using the co-registered water images. The kinetic rate constant K_i for FDOPA uptake at each voxel was calculated using the Patlak method with a cerebellar reference region as the input function. Within a VOI encompassing subcortical dopamine-rich regions, a voxel-wise regression analysis was performed with SPM5 to assess the relationship between presynaptic dopamine synthesis levels and the

four cognitive factors listed above. Voxel-wise correlations are reported at $p = 0.001$, uncorrected.

Results: For three of the cognitive factors, a positive correlation with dopamine synthesis (FDOPA Ki) was found in the following regions: processing speed, putamen, bilaterally (left: $r = 0.504$, $p = 0.0001$; right: $r = 0.521$, $p = 6.14e-5$), right caudate nucleus ($r = 0.445$, $p = 0.0001$), and ventral anterior nucleus of the right thalamus ($r = 0.477$, $p = 0.0002$); n-back, head of the left caudate nucleus ($r = 0.467$, $p = 0.0004$); card sorting, tail of the caudate, bilaterally (left: $r = 0.523$, $p = 5.73e-5$; right: $r = 0.543$, $p = 2.76e-5$) and left anterior and dorsomedial thalamic nuclei ($r = 0.486$, $p = 0.0002$).

Discussion: Discrete regional patterns of presynaptic dopamine synthesis were found to be associated with performance scores in three of the four separable cognitive domains. The anatomical specificity of our findings is not only in agreement with previous data on working memory⁶, but also suggests that various components of the dopamine system are related to different cognitive operations. Processing speed, the domain requiring greatest motor coordination, was found to correlate most robustly with dopamine synthesis in the nigrostriatal portion of the striatum and in the ventral anterior nucleus of the thalamus, which has a rich dopaminergic innervation in humans⁷. Working memory, requiring extensive processing by frontal and parietal association areas, was correlated with dopamine levels in the most cognitively-relevant portion of the striatum, the caudate nucleus. Card sorting was associated with dopamine synthesis not only in the caudate nucleus, but also in the left anterior and dorsomedial thalamic nuclei, which heavily project to prefrontal cortex and cingulate gyrus. Further work is needed to determine the nature of the modulatory effects of subcortical presynaptic dopamine on cortical and cognitive functioning in order to better interpret our observed associations. References: 1. Nieoullon A et al. Prog Neurobiol 2002;67:53 2. Gao W et al. PNAS 2003;100:2836 3. Bertolino A et al. Biol Psych 2008;64:226 4. Cropley V et al. Biol Psych 2006;59:898 5. Dickinson D et al Schizophr Bull 2010 Mar 29 6. Cools R et al. J Neurosci 2008;28:1208 7. Garcia-Cabezas M et al. Neuroimage 2007;34:965.

Disclosure: A. Ianni: None. J. Masdeu: None. D. Dickinson: None. P. Kohn: None. A. Benferhat: None. D. Eisenberg: None. D. Weinberger: None. K. Berman: None.

131. Limbic System White Matter Microstructure and Treatment Outcome in Major Depressive Disorder: a Large Scale DTI Study Using Legacy Data

Dan Iosifescu*, Wouter S. Hoogenboom, Roy H. Perlis, Jordan Smoller, Qing Zeng-Treitler, Vivian S. Gainer, Shawn N. Murphy, Susanne E. Churchill, Isaac Kohane, Martha E. Shenton

Mount Sinai Medical School and Harvard Medical School, New York, NY

Background: Abnormalities of limbic and prefrontal cortical networks crucial for emotional processing are reported in subjects with major depressive disorder (MDD). There is converging evidence suggesting frontal and temporal white matter abnormalities in MDD, but the number of large scale diffusion tensor imaging (DTI) studies is limited, in particular those that evaluate the impact of white matter abnormalities on treatment outcome.

Methods: A total of 108,275 medical records of patients with a billing diagnosis of MDD were selected from the electronic medical record systems of two major hospitals in Boston, MA. Natural language processing was subsequently applied to find one hundred and fifty subjects (92 with an MDD diagnosis and 58 healthy controls) with non-pathological brain MRIs which included DTI data. For all these subjects we then reviewed clinical charts to assign a treatment outcome status at the time of the MRI study. The image analysis sequence included computation of fractional anisotropy (FA) maps of the diffusion tensor, calculated using the software package 3DSlicer. We used an atlas-based white matter segmentation; the white matter atlas was coregistered to each subjects' FA map using FSL software. We used

multivariate analyses of covariance (MANCOVA) to compare the fractional anisotropy (FA) of the fornix and the cingulum bundle between study cohorts (which included control subjects and MDD groups defined by treatment outcome).

Results: The study population included one hundred and fifty subjects (MDD = 92, control = 58) with a mean age of 45.3 ± 15.4 years (aged 16–75 years, 55% female). MDD subjects with poor clinical outcome (i.e., those who failed to achieve remission over a 12 month period) revealed region selective reduced FA in limbic system white matter fibers, statistically significant for the medial body of the fornix ($F = 4.28$, $p < 0.01$, $\eta^2_p = 0.083$). Post-hoc analyses revealed that, compared to healthy control subjects, persistently depressed patients had 19.2% lower FA ($p = 0.009$), whereas partial responders and remitters had non-significant lower FA (12.5%, $p = 0.24$ and 12.3%, $p = 0.13$, respectively). Moreover, global and regional selective age-related FA decline was most notable for patients with persistent depression (r between -0.51 and -0.56, $p < 0.01$).

Discussion: Our data suggests that microstructural white matter abnormalities are present in the limbic system of MDD patients, in particular of those with persistent depression. Our findings contribute to the growing body of literature suggesting that specific brain microstructural white matter abnormalities underlie poor response to antidepressant treatments. With this study we also demonstrate the feasibility of investigating brain morphology in psychiatric populations using MRI data collected as part of routine clinical treatment.

Disclosure: D. Iosifescu: Part 2; Eli Lilly & Co., Pfizer, Inc., Reed Medical Education. Part 4; Aspect Medical Systems, Forest Laboratories, Janssen Pharmaceuticals. W. Hoogenboom: None. R. Perlis: Part 2; Eli Lilly & Co., AstraZeneca, Proteus Biomedical, Concordant Rater Systems. J. Smoller: None. Q. Zeng-Treitler: None. V. Gainer: None. S. Murphy: None. S. Churchill: None. I. Kohane: None. M. Shenton: None.

132. Valence-Specific Midbrain Dopamine Modulation of BOLD Response to Reinforced Emotional Cues

Mbemba Jabbi*, Philip Kohn, Raghav Mattay, Brett Cropp, Tiffany Nash, James Zhang, Jonathan S. Kippenhan, Karen Berman

National Institute of Mental Health, Bethesda, MD

Background: Avoidance is a central goal of aversive learning, whereas reward learning is signified by approach behavior. Therefore, successful avoidance behavior should precede possible aversive encounters whilst successful approach may overlap in time with rewarding experience. Such distinctive positive and negative motivational signals are likely to be, at least in part, conveyed by midbrain dopamine (DA) neurons that innervate mesocorticolimbic and nigrotriatal dopaminergic pathways[1]. However, the role of midbrain DA in modulating neural coding of predictive cues as opposed to the actual experience of aversive and rewarding outcomes is not known. In line with dopaminergic modulation of response to salient, motivational cues[1,2], we hypothesized more robust DA-modulated neural coding of negatively reinforced cues relative to outcomes, as opposed to enhanced neural coding of rewarding outcomes relative to reinforced cues[2].

Methods: Twenty-eight healthy participants underwent reinforcement[3,4] learning during event-related 3T fMRI (TR = 1.95s). Each trial consisted of 1) a video of a fearful, happy, or neutral facial expression (3 seconds); 2) a choice between two non-face pictures simultaneously presented (2.5 seconds), with one of the pictures portraying emotional content concordant with the preceding video; and 3) a 2.5 second outcome cue delineating either potential monetary reward if the concordant picture was correctly chosen, or potential loss if the non-concordant picture was chosen. Thus these trials occurred in three motivational contexts: positive (happy), negative (fearful), and neutral. Additionally, correct concordant choices were rewarded for positive context trials, or led to avoidance of loss for negative context

trials, in a probabilistic fashion 80% of the time. After preprocessing (8 mm smoothing) and first level analysis, random-effects analysis was performed to assess BOLD response by modeling the prediction and outcome of loss or reward. Finally, to test whether presynaptic DA synthesis [5] differentially predicts BOLD response to motivational signaling [3,4], 16 of the 28 fMRI participants underwent FDOPA PET. After SPM99 preprocessing, we defined a cerebellar gray matter reference region to calculate whole brain FDOPA Ki, reflecting presynaptic DA synthesis. A midbrain volume of interest was hand-drawn on native-space MRI images, and coregistered to the native-space FDOPA Ki images in order to extract average midbrain Ki values. These values were then used as covariates of BOLD response to predictive and outcome cues at $p < 0.001$ uncorrected.

Results: For loss-predicting fear cues, BOLD response was greater than neutral cues in the anterior insula, ventral and dorsal striatum, and cingulate and prefrontal cortex (PFC)/orbitofrontal areas bilaterally at $p < 0.05$ FDR corrected, while there was no such BOLD response at the time of outcome. Gain-predicting happy cues relative to neutral cues recruited left striatum and insula, as well as amygdala, ventromedial PFC and anterior cingulate cortices bilaterally at $p < 0.05$ FDR corrected, but to a lesser extent than at the time of gain outcomes, when there was a more robust activation of these regions bilaterally at $p < 0.005$ FDR corrected. Further, an inverse DA-BOLD coupling during negative trials was seen at the time of loss-predicting fear cues in the midbrain, right amygdala, superior temporal sulcus, and the left posterior cingulate, whereas at the time of aversive outcomes, midbrain DA positively modulated BOLD response in medial and lateral PFC regions. For positive trials, there was no relationship during predictive cues, but during gain outcome a DA-BOLD correlation was observed in the medial and lateral PFC, and negative coupling was found in the middle anterior cingulate.

Discussion: Behaviorally adapting to aversive and rewarding circumstances accordingly, is essential for survival in a complex environment. Our findings show a clear dissociation in BOLD response to aversive and reward-predicting cues in a network of regions implicated in coding stimulus salience. That is, during negative trials, fear cues produced more robust neural response than did loss outcomes. In contrast, for rewarded positive trials, more robust BOLD response was seen during outcome than during predictive cues. Additionally, we showed that these differential BOLD responses were predicted by midbrain DA levels, suggesting a crucial and complex DA involvement in behavioral adaptations. 1. Masayuki et al Nature 2009 2. Schultz J Neurophysiology 1998 3. Seymour et al Nature 2004 4. Pessiglione et al Nature 2006 5. Francois et al 1994.

Disclosure: M. Jabbi: None. P. Kohn: None. R. Mattay: None. B. Cropp: None. T. Nash: None. J. Zhang: None. J. Kippenhan: None. K. Berman: None.

133. Developmental Trajectory of Functional Activation Changes in Adolescents at Clinical High Risk for Schizophrenia

Katherine Karlsgodt*, Theo Van Erp, Carrie Bearden, Tyrone Cannon UCLA, Los Angeles CA

Background: Schizophrenia is considered to have a strong developmental component, with differences evident as early as childhood, and symptoms emerging across adolescence and young adulthood. The neural underpinnings of the ongoing developmental changes during this critical period may therefore be key for understanding the onset of schizophrenia. While structural magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI) studies have shown that there may be differences in the trajectories of brain development in both young patients with schizophrenia and adolescents at clinical high risk for psychosis compared with typically developing controls, it is as of yet unknown whether there are age-associated changes in functional MRI (fMRI) activation across this time period. We therefore sought to assess the trajectory of functional changes across adolescence using fMRI in a group of patients at clinical high risk for schizophrenia compared with a group of healthy volunteers.

Methods: We assessed a group of 19 healthy controls, and 20 patients determined to be at clinical high risk for developing schizophrenia (recruited through the UCLA Center for the Assessment and Prevention of Prodromal States; CAPPS). Our subjects ranged in age from 14-21 years old and we employed a cross-sectional analysis to investigate age-related changes. We administered a parametric Sternberg style verbal working memory task during fMRI to probe the working memory circuitry, which is thought to reside primarily in the frontal and parietal lobes. To control for performance differences, we calculated each subject's working memory capacity (using Cowan's formula), and analyzed the data for each subject only at the load closest to their own capacity. To test for an interaction between the relationship of age and functional activation, we modeled age for each group, and tested the relationship between the slopes for each group using a whole brain cluster corrected voxel-wise F-test, which was followed with t-tests to probe the directionality of the findings.

Results: Our voxel-wise analysis indicates that the slopes of the age-activation relationship significantly differ between clinical high risk participants and controls, specifically in left frontal (frontal eye field, dorsolateral prefrontal cortex, and Broca's area) and right parietal regions, which are areas known to be critical for working memory task performance. When this was decomposed with t-tests to determine the directionality of these interactions, healthy controls showed a predicted negative association between activation with age, while at-risk participants showed a significantly different pattern of increasing activation within the working memory circuitry with increasing age.

Discussion: Adolescents at clinical high risk for schizophrenia show a distinct pattern of age-related change in functional activation across adolescence relative to typically developing controls, indicating that the prodromal period may be associated with a deviation from the normal developmental trajectory in the ability to recruit cortical regions for use in working memory tasks. The age-associated decrease in activation in typically developing controls may represent a honing or maturing of the working memory circuitry into a more efficient network, a finding consistent with other work in healthy individuals. The increase found in the patient group may represent an emerging inefficiency leading up to disease onset. To our knowledge this is the first investigation of the relationship between fMRI activation and age across adolescence in high-risk subjects. Understanding the way in which such changes emerge over time may provide a better basis for interpreting differences seen in adult patients with schizophrenia, as well as helping to identify potential points for later intervention.

Disclosure: K. Karlsgodt: None. T. van Erp: None. C. Bearden: None. T. Cannon: None.

134. Chronic Cocaine Exposure Induces Putamen Glutamate and Glutamine Metabolite Abnormalities in Squirrel Monkeys

Marc Kaufman*, Xiaoxu Liu, J. Eric Jensen, Tim Gillis, Chun Zuo, Andrew Prescott, Melanie Brimson, Kenroy Cayetano, Perry Renshaw

McLean Hospital, Belmont, MA

Background: Clarifying mechanisms underlying chronic cocaine's brain effects may help develop effective cocaine abuse treatments. We used serial *in vivo* proton magnetic resonance spectroscopy (MRS) at 9.4 Tesla to characterize neurochemical effects of chronic cocaine exposure in squirrel monkeys. The putamen was selected because it exhibited time-dependent cocaine-induced dopamine transporter (Letchworth et al., *J. Neurosci.* 21:2799, 2001) and glucose utilization (Porriño et al., *J. Neurosci.* 24:3554, 2004) changes in macaques. We explored whether chronic cocaine exposure would induce time-dependent putamen proton metabolite changes.

Methods: Adult male squirrel monkeys ($N = 8$, 700-1000 g at study start) were split into saline control ($N = 4$) and cocaine exposure ($N = 4$) groups. Cocaine was administered intramuscularly (i.m.) 3 times/day, 5 days/week, for 9 months. MRS scans were acquired at baseline, before exposure to cocaine or i.m. saline and serially after 1, 3, 6, and 9 months of chronic cocaine/saline exposure, on a 9.4T Varian scanner with a STEAM sequence (TE/Mixing time

TM/TR of 9.7/7/4000 ms and 128 signal averages). The MRS voxel ($6 \times 6 \times 6 \text{ mm} = 0.216 \text{ cm}^3$) was positioned over the right putamen. For scanning, monkeys were sedated and intubated and maintained with 1.5-2% isoflurane gas. A circulating warm water blanket was used to maintain body temperature during scans and vital signs were monitored and maintained. Cocaine-exposed monkeys were initially administered 1 mg/kg/injections (1 week), followed by 2 mg/kg/injections (week 2), followed by 3 mg/kg/injections (45 mg/kg/week) for 8.5 months, simulating human weekly cocaine doses (Liu et al., *Neuropharmacol.* 18:243, 1998). Proton metabolites were quantified with LCModel using a GAVA simulated basis set, and expressed as metabolite/total creatine (tCr) ratios.

Results: Monkeys in both groups maintained body weights throughout the study. Cramer-Rao Lower Bounds (CRLBs) for all metabolites were <20%. Two-way (treatment, time) within-subjects repeated measures ANOVAs identified treatment x time interaction effects for glutamate (Glu/tCr, $F_{4,24} = 15.4$, $P < 0.0001$) and glutamine (Gln/tCr, $F_{4,24} = 3.0$, $P < 0.039$) metabolite ratios. Post-hoc pair-wise comparisons revealed that in cocaine-treated monkeys, 1-month Glu/tCr ratios were reduced from baseline ($t(3) = 4.91$, $P < 0.02$) and 6 and 9 month Glu/tCr ratios were increased from baseline ($t(3) = 5.35$, $P < 0.02$ and $t(3) = 9.81$, $P < 0.003$, respectively). Correlation analyses revealed a positive correlation between cocaine exposure duration and Glu/tCr ratio ($R^2 = 0.76$, $P < 0.0001$). Post-hoc pair-wise comparisons also revealed that in cocaine-treated monkeys, 9-month Gln/tCr ratios were increased versus cocaine group baseline ($t(3) = 4.73$, $P < 0.02$).

Discussion: We found time-dependent cocaine-induced changes in putamen Glu/tCr and Gln/tCr metabolite ratios. The glutamate effect and time course parallel anterior cingulate cortex glutamate metabolite findings in a human cross-sectional study involving subjects reporting 6-20 years cocaine use (Yang et al., *Psychiatry Res.* 174:171, 2009). Thus, our squirrel monkey model may have promise for studying chronic cocaine-induced glutamate metabolite abnormalities, which may reflect abnormal neuronal and glial glutamate compartmentalization. Given our findings and that certain cocaine abuse treatments under development are designed to correct glutamate abnormalities, including N-acetylcysteine and ceftriaxone (Kaliivas et al., *Neuropharmacol.* 56(S1):169, 2009; Knackstedt et al., *Biol. Psychiatry* 67:81, 2010), it appears that proton MRS and possibly other MRS modalities (e.g., carbon-13 MRS) may be useful for characterizing effects of chronic cocaine and how novel treatments alter glutamate compartmentalization. This study was supported in part by the Counter-Drug Technology Assessment Center (CTAC), an office within the Office of National Drug Control Policy (ONDCP), via Contract Number DABK39-03-C-0075 awarded by the Army Contracting Agency, by NIH grants S10RR019356, R01DA09448, and K02DA017324, by a grant from Varian, Inc., and by gifts from John and Virginia B. Taplin. The content of the information does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred.

Disclosure: M. Kaufman: Part 1; National Institutes of Health, Office of National Drug Control Policy, Varian Inc., GSK, Schering-Plough, Organon, Amgen, Novartis, NARSAD. Part 4; Varian Inc., GSK, Schering-Plough, Organon, NARSAD. X. Liu: None. J. Jensen: None. T. Gillis: None. C. Zuo: Part 1; National Institutes of Health. A. Prescott: None. M. Brimson: None. K. Cayetano: None. P. Renshaw: Kyowa Hakko, Novartis, GSK, Eli Lilly, Roche. Part 4; Eli Lilly, GSK, Roche.

135. GABA, Neuronal Synchrony, and Working Memory Performance in Schizophrenia

Lawrence Kegeles*, Chi-Ming Chen, Xiangling Mao, Arielle Stanford, Dikoma Shungu, Sarah H. Lisanby

Columbia University/New York State Psychiatric Institute, New York, NY

Background: Cognitive deficits in schizophrenia are a major source of disability in the illness but show only a limited response to

currently available treatments. Relationships among impaired working memory, disordered neuronal synchrony, and abnormal prefrontal GABA levels have been hypothesized in schizophrenia, and insight into these deficits may provide new directions for treatment development. According to this model, disruption of gamma band neuronal synchrony (1) may be secondary to impairment of dorsolateral prefrontal cortex (DLPFC) GABAergic neurons (2), and may contribute to cognitive deficits in patients with schizophrenia (3). The goal of this study was to acquire the novel combination of *in vivo* MRS measures of GABA together with high-density electroencephalograms (EEGs) indexing neuronal synchrony during a working memory task, offering the potential to further our understanding of the pathophysiology of working memory deficits in schizophrenia.

Methods: Twenty-three participants (11 patients and 12 healthy volunteers) enrolled in the study. Baseline resting-state EEGs of all 23 participants were recorded for at least 3 minutes with eyes open. These were followed by 34-minute EEG recordings while participants engaged in a modified Sternberg working memory task. High quality complete EEG recordings during the working memory task were obtained for eight healthy volunteers and six patients. MRS data for all 23 participants were collected on a separate day. GABA levels in the left DLPFC were measured with a 3.0 T GE MR system, using the volume-selective PRESS J-editing difference method with an 8-channel phased-array head coil (4) and quantified relative to the simultaneously acquired internal water signal of the DLPFC voxel.

Results: The hit rate for the patients was significantly lower than for healthy volunteers (two-tailed independent-samples t-test, $p = 0.026$). In the resting state, there was a significant relationship between GABA levels in the left DLPFC and power of gamma frequency band (30 to 56 Hz) for the anatomically corresponding scalp electrode (F3) (Pearson's correlation coefficient, $n = 23$, $r = 0.472$, $p = 0.023$). The relationship between GABA and gamma power was also examined in three stages of the working memory task: encoding, retention, and probe stages. All three stages showed a significant correlation between GABA and gamma power (two-tailed Pearson's correlation coefficient, $n = 14$: encoding stage, $r = 0.739$, $p = 0.003$; retention stage, $r = 0.688$, $p = 0.007$; probe stage, $r = 0.802$, $p = 0.001$). Patients had significantly lower gamma power than controls in every working memory stage (two-tailed independent-samples t-test, $n = 14$: encoding stage, $p = 0.022$; retention stage, $p = 0.024$; probe stage, $p = 0.024$). GABA was also correlated with the peak gamma frequency of the encoding stage (two-tailed Pearson's correlation coefficient, $n = 14$; $r = 0.546$, $p = 0.043$).

Discussion: The role of DLPFC GABA levels in subserving gamma band power both in the resting state and during a working memory task is supported by these data. The normal relationships between GABA and gamma band power appear to remain in schizophrenia, although working memory performance and gamma power are at lower levels in the illness. Further studies of additional cognitive functions and potential relations to GABA and gamma power in other brain regions might assist in further uncovering the mechanisms and circuitry of cognitive impairment in schizophrenia. This approach might inform the development of more specific treatments for this disabling aspect of the illness.

Financial support: Dana Foundation, Lieber Center for Schizophrenia Research, Irving Center for Clinical Research

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with an 8-channel phased-array head coil at 3.0 T in the human dorsolateral prefrontal cortex using the J-editing technique. Proc Int Soc Mag Reson Med 2006; 14:488.

Disclosure: L. Kegeles: Part 1; Pfizer, Amgen. Part 4; Pfizer, Amgen. C. Chen: None. X. Mao: None. A. Stanford: None. D. Shungu: None. S. Lisanby: Part 1; Northstar DSMB, Astra Zenica. Part 4; ANS/St. Jude Medical, Neuronetics, Cyberonics, Brainsway, Magstim, Magventure.

136. Shared and Differential Patterns of Amygdalo-Cortical Activation Across Anxiety Disorders

William Killgore*, Jennifer Britton, Isabelle Rosso, Zachary Schwab, Melissa Weiner, Scott Rauch

Harvard Medical School, Belmont, MA

Background: Anxiety disorders represent a category of psychiatric disorders that involve apprehension about potential future harm or aversive situations. Although anxiety is a shared core feature of all of these disorders, there are also differences in clinical criteria that distinguish among them. Neuroimaging research has identified several interconnected brain structures that respond to threatening stimuli, and which respond differently in patients with various anxiety disorders relative to healthy controls. Notably, patients with PTSD show a pattern of reduced activation within the ventromedial prefrontal cortex (VMPFC) and exaggerated responses within the amygdala. Unknown, however, is the degree to which various anxiety disorders share a common pattern of responsiveness within the threat assessment system and whether these disorders can be distinguished based on differential responsiveness of this system to affective stimuli. Accordingly, we compared brain activation patterns across four groups of individuals meeting diagnostic criteria for post-traumatic stress disorder (PTSD), panic disorder (PD), or specific animal phobia (SP), and healthy controls (HC) during functional magnetic resonance imaging (fMRI) using an established probe of cortico-limbic function. It was hypothesized that anxiety disorders as a group would show reduced activation within VMPFC and exaggerated activation within limbic/paralimbic regions including the amygdala and insula. Further, based on clinical phenomenology of the disorders and preliminary data, we hypothesized that the activation patterns would cluster into two groups. Specifically, PTSD and PD were hypothesized to share a common generalized threat response (GTR) involving reduced VMPFC and increased amygdala responses to nonspecific threat cues (i.e., masked fearful faces). In contrast, SP subjects were expected to show only disorder specific threat responses, and would therefore, not differ from HC subjects in the responses of these regions to the masked faces paradigm.

Methods: Fifty-five adults (15 PTSD; 12 PD; 11 animal phobia; 17 non-psychiatric controls) underwent fMRI (3T, TR = 2 sec, TE = 30 msec, flip angle = 90 degrees) while engaged in a backward masked fear vs. neutral face passive viewing paradigm. In a block design, 12 epochs (28 seconds each) of backward-masked faces (16 msec affective face target; 184 neutral face mask) were presented at a rate of two target-mask pairs per second. The task alternated blocks of masked happy, masked fear, and masked neutral expressions with epochs of low-level fixation crosshair. Two runs of the task were administered, each lasting 336 seconds, with order counterbalanced across sessions. Within SPM5, data were motion corrected, normalized to the 3-dimensional MNI space, and smoothed using an isotropic Gaussian kernel (FWHM = 6 mm), and data were corrected using AR(1) and a low frequency drift was removed with a high pass filter of 128 seconds. Masked Fear and Neutral conditions were contrasted with one another. Diagnostic groups were compared using a one-way ANOVA, followed by planned comparisons (e.g., HC vs all anxiety groups). All data were evaluated at a whole brain level, $p < .005$ (uncorrected), with a cluster size (k) of 5 contiguous voxels.

Results: Relative to the HC group, the combined anxiety disorder sample (SP + PTSD + PD) showed significantly reduced activation

within the VMPFC and greater activation within the left amygdala and left insula. Similarly, a planned comparison between GTR (PTSD + PD) and control (HC + SP) groups showed that the GTR group exhibited comparatively reduced activation within the VMPFC and increased activation within the left amygdala and bilateral insula relative to the control group. Post-hoc comparisons supported the hypothesis that the PTSD and PD groups were similar to one another in the responsiveness of these brain regions, but differed from healthy (HC) and psychiatric (SP) control groups, which were generally similar in responsiveness.

Discussion: As a group, anxiety disorders were distinguishable from HCs by a common pattern of reduced VMPFC and increased limbic/paralimbic activation to an established masked affect probe. Moreover, this pattern appeared to be specific to the disorders of PTSD and PD, which involve GTR, whereas the SP group was virtually indistinguishable from HCs in the responsiveness of these regions. Findings suggest that anxiety disorders appear to share a common pattern of corticolimbic responses, but are also distinguishable from one another based on the magnitude of these responses to general (disorder non-specific) threat stimuli.

Disclosure: W. Killgore: None. J. Britton: None. I. Rosso: None. Z. Schwab: None. M. Weiner: None. S. Rauch: *Part 1*; Vanderbilt University – honorarium, VA Roundtable - consult, Oxford Press - royalty, APPI – royalty, Wolters Kluwer – royalty, ACNP - honorarium, Flinders University – honorarium, UTSW Dallas Grand Rounds. *Part 4*; Cyberonics, Medtronics, Northstar Neuroscience.

137. Neurocircuitry of Emotional Regulation in Veterans Returning from Iraq and Afghanistan with and Without PTSD: Effects of Diagnosis and Genotype

Anthony King*, Sarah Garfinkel, Rebecca Sripada, Xin Wang, Alexander Taylor, Israel Liberzon

University of Michigan, Ann Arbor, MI

Background: Posttraumatic stress disorder (PTSD) is associated with exaggerated emotional responses and deficits in emotional regulation. Neuroimaging studies show combat PTSD patients have increased amygdala responses and decreased medial prefrontal cortex (mPFC) activity during trauma-related as well as general aversive emotional stimuli. Risk for developing PTSD after trauma exposure has clear genetic components. Although PTSD genetics are not well understood, preliminary findings link specific variants (e.g. serotonin transporter promoter polymorphism 5-HTTLPR, SNPs in the FKBP5 gene) to PTSD symptoms. The 5-HTTLPR “short” allele is also associated with elevated amygdala responses to aversive emotional stimuli, suggesting a potential mechanism for altered emotional processing that could contribute to psychiatric vulnerability.

Methods: Veterans returning from combat deployments to Afghanistan and Iraq (OEF and OIF, respectively) seeking treatment for PTSD ($n=14$) and healthy veterans without PTSD history ($n=10$) were recruited from the VA Ann Arbor. Healthy controls (no PTSD history, matched by age, education, income), were recruited from the community ($n=11$). All subjects received psychiatric diagnostic interview (MINI). PTSD subjects met DSM-IV criteria for PTSD and CAPS scores > 50 . DNA was obtained from saliva; tri-allelic genotype on serotonin transporter promoter (5-HTTLPR) polymorphism was determined by PCR followed by HpaII digestion. Emotional regulation neuroimaging paradigms were performed in a 3T fMRI environment. We utilized aversive and neutral complex social scenes from the International Aversive Picture System (IAPS) as emotional induction stimuli and control conditions. Pictures were presented in blocks of 4 pictures (4 sec each) interspersed with blocks of fixation cross. In the “unregulated” emotional response (“Just View” condition), subjects were instructed to view the pictures and to maintain the evoked emotional response. During the emotional regulation (“Rate”) condition, subjects viewed pictures while noticing and rating negativity of their emotional response.

Results: We first examined effects of our emotional induction and regulation paradigms in the entire cohort ($n=35$). Viewing aversive pictures activated visual cortex, ventral limbic areas, amygdala and hippocampus, bilateral dlPFC, and dmPFC, effects seen in each group separately. Contrasts of “Rate” aversive vs. “View” aversive conditions in the entire cohort found significant (whole brain threshold $p < .001$) activation of a wide area of the medial frontal wall (dmPFC, dACC, and rostral ACC) and bilateral caudate, and similar activations of dmPFC/dACC and caudate were found in each group separately. Effects of PTSD diagnosis, combat exposure, and 5-HTTLPR genotype were examined in between group contrasts. PTSD patients had greater (whole brain $p < .005$) activation of bilateral hippocampus, parahippocampus, and amygdala during “Viewing” of neutral IAPS pictures compared to both control groups, and while “Viewing” aversive pictures PTSD patients had greater activation of perigenual ACC than combat controls and less activation of dmPFC than non-deployed controls. PTSD also had greater activation of hippocampus in the “Rate” > “View” contrast than controls. In regard to 5-HTTLPR genotype, alleles were evenly distributed across group (3 LA/LA each in PTSD, combat controls, and non-deployed controls). Contrast of S-carriers ($n=20$) vs. LA/LA ($n=9$) found S-carriers had greater activity in bilateral posterior insula, while LA/LA had greater activity in rostral ACC (BA 24)/rPFC (BA9); effects also seen in groups separately.

Discussion: These data suggest returning military veterans with PTSD have relatively greater activity in limbic regions (amygdala and hippocampus) while viewing normatively rated “neutral” social stimuli, and while engaged in rating of emotional responses. Serotonin transporter gene 5-HTTLPR S-allele carriers have greater activity in emotional activation (insula) and less activity in regulatory (rACC, PFC) regions. We are continuing analyses examining interaction of combat exposure with genotype.

Disclosure: A. King: *Part 1*; VA Ann Arbor Healthcare System. S. Garfinkel: None. R. Sripada: None. X. Wang: None. A. Taylor: None. I. Liberzon: None.

138. Physiological and Behavioral Effects of SYN-115, a Novel Adenosine2A Antagonist in Cocaine Dependent Subjects.

Kimberly Kjome*, Frederick Moeller, Joel Steinberg, Stephen Bandak, Scott Lane, Liang suo Ma, Nuvan Rathnayaka, Larry Kramer, Ponnada Narayana

University of Texas-Houston Medical School Department of Psychiatry and Behavioral Science, Houston, TX

Background: Adenosine2A receptor antagonists have been shown to enhance dopamine function, and novel drugs in this category are being developed for use in conditions such as Parkinson’s disease. Cocaine dependence is associated with reduced dopamine function and dopamine-enhancing medications have shown promise as potential treatments for cocaine addiction. SYN-115 is a novel adenosine2A antagonist currently being studied for Parkinson’s disease. The purpose of this study was to examine the behavioral and physiologic effects of SYN115 in cocaine dependence.

Methods: 19 cocaine dependent subjects received doses of SYN115 (100 mg) or placebo. At fixed intervals before, during, and after dosages were given, patients received measurements of blood pressure and heart rate, and completed visual analog scales measuring subjective effects bad or good effects of drug, and drug liking.

Results: 14 subjects received SYN 115 and 5 subjects received placebo. One way repeated measures ANOVA compared systolic, diastolic and heart rate measurements over 4 time points corresponding to medication doses with placebo and placebo vs. SYN 115. There was no significant interaction between drug (placebo vs. SYN115) and time (pre vs. post dose) for systolic blood pressure ($F = 0.24$, $p = 0.92$) or heart rate ($F = 1.63$, $p = 0.176$). However, there was a significant interaction between drug and time for diastolic blood pressure ($F = 0.276$, $p = 0.034$), with mean diastolic blood pressure being higher

in SYN115 treated subjects (76.4 ± 11.0) than placebo treated subjects (70 ± 5.0), largely due to a reduction in blood pressure in placebo treated subjects. T-tests on visual analog scales showed significantly higher scores in SYN 115 dosing for feeling effects of drug, and rated it as something they would take again, but with one-way repeated measures ANOVA, these scales were not significant compared to baseline scores. There was no significant difference between SYN115 and placebo for bad effects or sedation. There were no significant adverse effects of SYN115.

Discussion: These preliminary results show that the selective A2A antagonist SYN115 is well tolerated in cocaine dependent subjects, showing a statistically but not clinically significant increase in diastolic blood pressure but no significant effects on systolic blood pressure or heart rate. Subjects rated SYN115 as having a subjective drug effect but did not rate the SYN115 as having bad effects, although this effect was not significant after correcting for baseline ratings.

Disclosure: K. Kjome: None. F. Moeller: None. J. Steinberg: None. S. Bandak: Part 5; Synosia Pharmaceuticals, San Francisco CA. S. Lane: None. L. Ma: None. N. Rathnayaka: None. L. Kramer: None. P. Narayana: None.

139. Cortisol Awakening Response and Cerebral Serotonin Transporter Binding

Gitte M. Knudsen*, Vibe G. Froekjaer, David Erritzoe, Peter Jensen, Jacob Madsen, William Baaré

Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark

Background: Stress sensitivity and serotonergic neurotransmission seem to interact, e.g. in relation to vulnerability for mood disorders. In particular, an increased physiological stress response is genetically linked to the serotonin system through the low expressing s-variant of the 5-HTLPR polymorphism in the promoter region of the serotonin transporter (SERT) gene. The HPA-axis activity can be characterized by the cortisol rise following wakening; the cortisol awakening response. In this study we investigated whether HPA-axis activity is associated with cerebral serotonin transporter binding and whether this association might be modified by the s and l genotype of the SERT promoter.

Methods: Thirty-two healthy volunteers (mean age 35 ± 20 , 7 women) underwent serotonin transporter imaging with ^{11}C [DASB]-PET and performed home-sampling of the cortisol awakening response (CAR). CAR was described by two variables: 1) Area under the curve with respect to ground (AUCg) that primarily represents the total hormonal output, and 2) AUC with respect to increase (AUCi) that emphasizes changes over time and thus represents more the HPA-axis reactivity. We tested whether CAR predicted neocortical, pallidostriatal, or frontal SERT binding in a multiple linear regression model with adjustment for age. Interactions between CAR measures and s-allele carrier/non-carrier status were also tested.

Results: The stress reactivity component of CAR (AUCi) correlated positively with frontal SERT ($p = 0.02$) and also tended to correlate positively with pallidostriatal SERT ($p = 0.07$). On the other hand, the total cortisol output component (AUCg) correlated positively with pallidostriatal SERT ($p = 0.04$), whereas it did not correlate significantly with frontal SERT ($p = 0.10$). No significant interaction between CAR variables and the s-allele carrier/non-carrier status in predicting SERT binding was present. Also, no significant main effect of s-allele carrier/non-carrier status on SERT binding could be demonstrated.

Discussion: Our findings support, that high SERT binding in frontal regions is associated with larger HPA-axis reactivity in mentally healthy individuals. Also, pallidostriatal SERT seemed to be positively associated with both total cortisol output and reactivity of the HPA-axis. Even though an association between CAR and the low-expressing variant of the SERT promotor gene is well documented, our data suggests that this genotype does not modify

the association between cerebral SERT binding and CAR in mentally healthy individuals.

Disclosure: G. Knudsen: None. V. Froekjaer: None. D. Erritzoe: None. P. Jensen: None. J. Madsen: None. W. Baaré: None.

140. Connectivity of Brain Structures Correlates with Treatment Outcome in Major Depressive Disorder

F. Andrew Kozel*, Uma Rao, Hanzhang Lu, Paul Nakonezny, Bruce Grannemann, Tamara McGregor, Paul Croarkin, Kimberly Mapes, Carol Tamminga, Madhukar Trivedi

UT Southwestern Medical Center, Dallas, TX

Background: Major Depressive Disorder (MDD) is a serious brain syndrome that causes considerable morbidity and mortality. The identification of biosignatures to assess the probability of response to an antidepressant for patients with Major Depressive Disorder (MDD) is critically needed. Functional MRI (fMRI) offers the promise to provide such a measure. Previous work with non-task fMRI (also referred to as resting state fMRI) has demonstrated that the correlation in signal from one region to another is a measure of connectivity. This study investigated whether measures of connectivity between brain regions prior to treatment could be significantly correlated with clinical treatment outcome. This could lead to a feasible diagnostic test that would enable clinicians to know the likelihood that a patient with MDD would respond to a particular treatment.

Methods: In this pilot work, participants with non-psychotic MDD and no significant co-morbidities were recruited from the community and other research protocols. After informed consent was obtained, participants underwent screening and clinical evaluation. A baseline non-task fMRI was subsequently obtained. Participants were free of all medications when scanned. Images were acquired using a research-dedicated 3T Philips Achieva scanner (Philips Medical System, Netherlands) with an eight-channel SENSE head coil. Participants were instructed to hold still, keep their eyes open, and focus on a cross in the middle of the screen. The echo-planar imaging non-task fMRI scan was obtained with the parameters of TR 2000 ms, 44 slices, 3 mm slices, FOV 220x132x220, matrix 64x64, with resulting voxel dimension of 3.4375x3x3.4375 mm³. There were 240 time points with a total scan time of 502 sec (8 min 22 sec) that included dummy and saturation scans. After scanning was completed, participants were treated with an antidepressant outside the study protocol. After 8 weeks of treatment, they were clinically re-evaluated. MRI data analysis was performed using FSL. Probabilistic anatomic regions of interest (ROI) were defined for 16 brain regions previously identified as being important in mood disorders (eight for each hemisphere - amygdala, hippocampus, anterior cingulate gyrus, posterior cingulate gyrus, medial frontal cortex, orbitofrontal cortex, middle frontal cortex, and subcallosal cortex). These ROIs were used to determine mean time courses for each individual's baseline non-task fMRI. Measures of connectivity were then determined by estimating the Spearman rank-order correlation coefficient between the time-course of all 16 ROIs for each individual. These calculated correlations were considered to be measures of connectivity. The degree of connectivity was tested to determine which ones significantly correlated with treatment outcome. The primary treatment outcome was defined as the percent change in QIDS-SR from baseline to week 8 with a response being defined as a 50% improvement.

Results: Thirteen (10 female, mean age 33.7, s.d. 7.4, range 22-48 years) of the seventeen participants enrolled had adequate imaging and clinical data. Ten participants took bupropion SR 150 mg twice a day as part of a clinical trial, two took escitalopram 20 mg once a day, and one took aripiprazole 5 mg once a day. Seven of the 13 who were evaluated at eight weeks met criteria for response. Connectivity measures in several regions, especially the subcallosal cortex, were robustly correlated with treatment outcome. As an example of the potential ability to provide important clinical information, choosing a connectivity value of less than 0.1 for the left subcallosal

cortex to the left anterior cingulate as a predictor of treatment response, 11 of the 13 participants (85% accuracy) would have had their treatment outcome correctly ascertained prior to treatment. A one-sample test here of the binomial proportion (H_0 : Proportion = 0.5) revealed rejection of the null hypothesis (two-sided exact test, $p = 0.02$).

Discussion: Connectivity measures of specific brain regions during a pretreatment non-task fMRI could provide a means to evaluate how likely a patient is to respond to an antidepressant treatment. Further work is required to confirm these findings in larger samples and to assess if these measures of connectivity can be used to predict differential outcomes among two or more antidepressant treatments.

Disclosure: F. Kozel: Part 4; Cephos Corp, Neuronetics for Grant-in-Kind. U. Rao: None. H. Lu: None. P. Nakonezny: None. B. Grannemann: None. T. McGregor: None. P. Croarkin: None. K. Mapes: None. C. Tamminga: Part 1; Acadia Pharmaceuticals, Inc. - Advisory Board, drug development, American Psychiatric Association - Deputy Editor, Amylin - *Ad hoc* consultant, ARYx Therapeutics - *Ad hoc* consultant, Astellas Pharma US, Inc. - Consultant, Astra Zeneca - Consultant, Avera - Advisory Board, drug development, Becker Pharma - *Ad hoc* consultant, Eli Lilly - *Ad hoc* consultant, Finnegan Henderson Farabow Garrett & Dunner, LLP - Expert Witness, International Congress on Schizophrenia Research - Organizer, Intracellular Therapies (ITI, Inc.) - Advisory Board, drug development, Lundbeck, Inc. - *Ad hoc* consultant and speaker, Neurogen - Advisory Board, drug development, Nupathe - consultant, drug development, Orexigen - *Ad hoc* consultant, Organon - *Ad hoc* consultant, Otsuka - Consultant, Saegis - *Ad hoc* consultant Sumitomo - *Ad hoc* consultant, Zogenix - Consultant. Part 2; American Psychiatric Association - Deputy Editor, Intracellular Therapies (ITI, Inc.) - Advisory Board, drug development, Lundbeck, Inc. - *Ad hoc* consultant and speaker, Finnegan Henderson Farabow Garrett & Dunner, LLP - Expert Witness, Astellas Pharma US, Inc. - Consultant, Patterson, Belknap, Webb & Tyler (for Johnson & Johnson) - One time consultant on a risperidone patent case, Intracellular Therapies - Advisory Board, drug development Avera - Advisory Board, drug development, Neurogen - Advisory Board, drug development. Part 4; Acadia Pharmaceuticals, Inc. Amylin Pharmaceuticals, Inc. M. Trivedi: Part 1; Abbott Laboratories, Inc. Abdi Ibrahim Akzo (Organon Pharmaceuticals Inc.) AstraZeneca Axon Advisors Bristol-Myers Squibb Company Cephalon, Inc. CME Institute of Physicians Cyberonics Inc., Eli Lilly & Company, Evotec, Fabre Kramer Pharmaceuticals, Inc. Forest Pharmaceuticals GlaxoSmith-Kline Janssen Pharmaceutica Products, LP Johnson & Johnson PRD Lundbeck Meade Johnson MedAvante, Medtronic Neuronetics Otsuka Pharmaceuticals Parke-Davis Pharmaceuticals, Inc. Pfizer Inc. PGxHealth Rexahn Pharmaceuticals Sepracor SHIRE Development Solvay Pharmaceuticals VantagePoint Takeda, Wyeth-Ayerst Laboratories. Part 4; Agency for Healthcare Research and Quality Corcept Therapeutics, Inc. Cyberonics, Inc. Merck National Alliance for Research in Schizophrenia and Depression National Institute of Mental Health, Predix Pharmaceuticals (Epix) Solvay Pharmaceuticals, Inc. Targacept.

141. Differential Patterns of Abnormal Neural Activity and Functional Connectivity to Emotional Faces in Bipolar and Bipolar NOS Youth

Cecile Ladouceur*, Patrick Pruitt, Vaibhav Diwadkar, Tiffany Farchione, David Axelson, Boris Birmaher, Jacqueline Radwan, Mary Phillips

University of Pittsburgh, Pittsburgh, PA

Background: Bipolar disorder (BP) is a devastating neuropsychiatric illness that affects 2-5% of the population. Childhood-onset BP may be a particularly severe form of the illness. Youth with subthreshold symptoms of BP, such youth diagnosed with BPNOS, are a particularly vulnerable group as evidence suggests that a high percentage will convert to BPI/BPII, yet very little is known as to

which markers (clinical, biological) predict this subsequent conversion to BP. Recent neuroimaging studies suggest that BPI is associated with functional abnormalities in neural systems supporting emotion processing and emotion regulation. In this study, we examined whether youth with BPNOS will exhibit abnormal activity in, and connectivity between, regions in neural systems underlying emotional processing and emotion regulation, and the extent to which these abnormalities are in common with neural system abnormalities in BPI youth.

Methods: A total of 52 children and adolescents (8-17 years old) participated in the study. Of these, 18 met DSM-IV criteria for bipolar disorder type I (BPI), 16 had sub-threshold symptoms of BP (BPNOS), and 18 were age- and sex-matched healthy controls (HC). Neural activity was measured using fMRI while participants completed two tasks that involved identifying the gender of actors displaying emotional facial expressions (happy/neutral and fearful/neutral). fMRI blood oxygen level dependent signal change was collected using a 3T scanner. fMRI data were processed and analyzed using SPM5. Second-level random-effects group analyses were conducted on first-level t-contrasts and focused on the 3 (group) by 2 (valence) interaction for each task (happy, fearful), covarying for age, sex, and behavioral performance. Region-of-interest (ROI) analyses were performed on the following regions: amygdala, ventromedial prefrontal cortex (VMPFC), and dorsolateral prefrontal cortex (DLPFC). AlphaSim was used to correct for family-wise error (FWE) and determine the cluster threshold at $p < .05$. Functional connectivity analyses were performed in SPM5 using VMPFC as a seed region.

Results: For the happy face task, there was a significant group by valence interaction for all three ROIs. Post hoc analyses revealed that compared to HC, BPI exhibited greater activation in amygdala, VMPFC, and DLPFC to happy faces. BPNOS, compared to HC, also exhibited greater activation in VMPFC and DLPFC to happy faces, but this effect was a trend ($p = .10$). For the fearful face task, there was a significant group by valence interaction for DLPFC. Post hoc analyses revealed that compared to HC, BPI, but not BPNOS, exhibited reduced activation in DLPFC to fearful faces. Findings from the functional connectivity analyses on the happy face task suggest that compared to HC, BPNOS, but not BPI, showed greater connectivity between VMPFC and DLPFC to happy faces. Furthermore, findings on the fearful face task revealed that compared to HC, BPI, but not BPNOS, showed reduced connectivity between VMPFC and amygdala to fearful faces.

Discussion: These findings indicate that BPNOS and BPI exhibit different patterns of abnormal neural activity and functional connectivity while processing emotional facial expressions. Future longitudinal studies are needed to elucidate the neurodevelopmental trajectories of connectivity in neural systems supporting emotion processing and emotion regulation in BPI and BPNOS youth and the extent to which abnormalities in these trajectories represent biomarkers of risk for future conversion to BP in BPNOS youth.

Disclosure: C. Ladouceur: None. P. Pruitt: None. V. Diwadkar: None. T. Farchione: None. D. Axelson: None. B. Birmaher: None. J. Radwan: None. M. Phillips: None.

142. An fMRI Investigation Of Temporal Discounting In Schizophrenia

Adrienne Lahti*, Kathy Avsar, David White, Meredith Reid, Mark Bolding, Luke Stoeckel, Rosalyn Weller, James Cox

University of Alabama at Birmingham, Birmingham, AL

Background: The aim of this study is to evaluate elements of reward processing in schizophrenia (SZ), and more specifically, whether the neural correlates of temporal discounting are impaired in the illness. The construct of temporal discounting is based on the observation that as the delay of a reward delivery increases, the valuations of this reward decreases. Several aspects of reward

processing, including reward anticipation and delivery, attribution of salience, reinforcement learning, and prediction error have been found to be abnormal in SZ. Barch & Dowd (2010) recently proposed a neuronal model integrating key elements of reward processing, including attribution of hedonic value, reward prediction and reinforcement learning, information integration-effort allocation, and action plan towards valued outcome. Temporal or delay discounting, while not tapping into reward prediction or reinforcement learning, maps onto elements of value-information integration. Indeed, imaging studies of temporal discounting show that making delayed over short term choices is driven by the prefrontal networks (McClure et al, 2004). In addition, Heerey (2007) has observed that the steeper discounting seen in SZ vs. healthy controls (HC) was related to worse memory capacity, suggesting abnormal value representation. In this fMRI study, we compared SZ and HC during performance of a delayed discounting (DD) task. We had hypothesized that abnormal BOLD response in SZ would be observed in neuronal networks encompassing the midbrain, the striatum, the orbitofrontal cortex (OFC), and the dorso- (DLPFC) and ventrolateral- (VLPFC) prefrontal cortex.

Methods: A 3T head-only Siemens Allegra magnet was used to acquire scans in stable, medicated SZ (SZ; n = 8; mean age = 39 ± 14 years, 5 M/3 F) and matched HC (HC; n = 9; mean age = 39 ± 14, 5M/4F). The DD task consisted of 4 sessions of 40 trials each. Each trial consisted of a choice between a smaller monetary immediate reward and a larger delayed reward. Prior to scanning, the discounting rate (k) that provided an equal number of immediate and delayed choices was determined for each participant. This optimized discounting rate was used during scanning acquisition. The acquisition parameters were as follows: TR = 2.2 sec, TE = 30 ms, Flip angle = 70 degrees, slice thickness = 4 mm, 1 mm gap, 30 slices. Single-shot gradient echo, echo-planar imaging was used. Data were preprocessed using SPM8 (slice timing correction, motion correction, and when appropriate, normalization to MNI coordinate space and smoothing). The fMRI data were analyzed using standard whole-brain mixed-effects analysis implemented in SPM8. The following contrasts were modeled as a time series of discrete events that were convolved with the canonical hemodynamic response function and the resulting temporal derivative: hard vs. easy choices and immediate vs. delayed choices. The statistical threshold was set at p < 0.005 with a minimum of eight contiguous voxels.

Results: Within group analysis: When the HC made hard compared to easy choices, they showed greater BOLD response in the substantia nigra (SN), the left parahippocampus, and the right DLPFC, and smaller response in the ventral striatum (VS) and subgenual anterior cingulate cortex (ACC). When the HC made delayed compared to immediate choices, they showed greater BOLD response in the left hippocampus, and the thalamus. Between group analysis: In contrast to SZ, HC showed greater BOLD response in the midbrain, left parahippocampus and right DLPFC in the contrast hard vs. easy choices, and greater response in the ACC and right DLPFC in the contrast of delayed vs. immediate choices.

Discussion: These preliminary data demonstrate changes in the temporal discounting-induced BOLD response in regions previously identified in discounting tasks. In addition, we found differences in BOLD signal between the SZ and the HC in a network encompassing the midbrain, the ventral striatum, and the prefrontal cortex. Studying differences in the neural correlates of temporal discounting in schizophrenia could lead to a better understanding in the impairments in drive and goal-directed behaviors associated with the illness. Barch, D. M., & Dowd, E. C. (2010). Goal Representations and Motivational Drive in Schizophrenia: The Role of Prefrontal-Striatal Interactions. *Schizophr Bull.* 36:919-34.

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Disclosure: A. Lahti: Part 1; Pfizer, Lunbeck. Part 2; Pfizer. Part 4; Pfizer, Janssen. K. Avsar: None. D. White: None. M. Reid: None. M. Bolding: None. L. Stoeckel: None. R. Weller: None. J. Cox: None.

143. Post-Error Slowing In MDD And 5-HTTLPR Genotype

Scott Langenecker*, Sara Walker, Kathleen Hazlett, Srijan Sen, Brian Mickey, Rachel Kay, Kortni Meyers, Amy Ransohoff, Michael Ransom, Susan Kennedy, David Hsu, Huda Akil, Douglass Noll, Elizabeth Young, Margit Burmeister, Jon-Kar Zubieta

University of Michigan, Ann Arbor, MI

Background: An important new area of research in mood disorders is error monitoring, yet little research has been conducted on error monitoring and post error slowing (PES) in those with MDD to date. In addition, there is a suggestion that cognitive problem solving and regulation may be mediated by a functional variant in the serotonin transporter (5-HTT) gene, 5-HTTLPR. There is also evidence to suggest that 5-HTTLPR may be associated with increased risk for MDD under stress, and we hypothesize that cognitive set-shifting and regulation may be the underlying neural mechanism responsible for the increased risk of mood disorders in those with lower functioning 5-HTTLPR alleles.

Methods: Individuals with SCID-IV verified MDD (n = 24) were compared to healthy control subjects (n = 28) recruited for a study using the Parametric Go/No-Go Task completed during fMRI (Langenecker et al., 2007). The mean Hamilton Depression Score for the MDD group was 19. Genomic DNA was extracted from blood and the 5-HTTLPR variant was genotyped using standard methods and consistent with prior work by our group (Sen et al., 2004). Reaction time to targets and errors were the primary dependent variables. Commission errors could be present on either the 2 or the 3 target Go/No-go levels of the task. Specific response times were then calculated based upon relative position of targets in relation to errors (pre-1 error, error, and post 1 error through post-5 error position). Alternatively, post-error slowing was calculated using reaction times between 4 seconds prior to and 8 seconds after the average error. As stimuli are rapidly presented in the PGNG, PES can be calculated on a 500 ms resolution scale.

Results: A repeated measures ANOVA demonstrated that MDD diagnosis (p < .001) and number of 5-HTTLPR low-functioning (s) alleles (p = .013) were both associated with longer reaction time on the 3 target Go, 2 target Go/No-go, and 3 target Go/No-go levels.). A second repeated measure ANOVA with reaction time for errors at each of six positions (error through post 5 error) showed a significant interaction between position, number of s alleles, and Group (p = .009). There was also a main effect of group (p = .04), but not for 5HTTLPR genotype (p = .15). Additional posthoc analyses indicated that the greater post-error slowing in MDD was primarily in the l/s group. The MDD s/s group was just slower at each position, including the error itself. The s/s control group also showed a large PES effect. The l/l MDD and both l allele control groups showed similar PES effects. Preliminary brain imaging results showed that the PES was significantly positively correlated with activation in bilateral rostral anterior cingulate gyrus and inversely correlated with activation in the right middle frontal gyrus.

Discussion: The present results suggest that abnormal error processing may be mediated by 5-HTTLPR and exacerbated by the presence of MDD. It is possible that hypersensitive regulatory mechanisms work well in situations with low stress, yet may be easily overwhelmed in high demand or high stress situations. Future work with those at risk for MDD may disentangle whether these PES effects are a risk for, or are a result of depression.

Disclosure: S. Langenecker: None. S. Walker: None. K. Hazlett: None. S. Sen: None. B. Mickey: None. R. Kay: None. K. Meyers: None. A. Ransohoff: None. M. Ransom: None. S. Kennedy: None. D. Hsu: None. H. Akil: None. D. Noll: None. E. Young: None. M. Burmeister:

None. J. Zubieta: Part 1; Eli Lilly, Merck. Part 2; Eli Lilly. Part 3; Eli Lilly.

144. Heritability of White Matter Microstructure Assessed Using the Tensor Distribution Function

Alex Leow*

University of Illinois, Chicago, IL

Background: Fractional anisotropy (FA), a measure of white matter integrity derived from the diffusion tensor, is highly heritable, and there is a genetically-mediated correlation between fiber integrity and intellectual performance.

Methods: To study this further, we investigated the heritability of the white matter microstructure of the human brain, using the Tensor Distribution Function (TDF). This new technique exploits the angular detail in high angular resolution diffusion imaging (HARDI) to correctly compute fractional anisotropy where fibers mix or cross, overcoming errors in the single-tensor model. We scanned 29 pairs of monozygotic twins (MZ; 30 men/28 women; $25.1 \pm 1.7\text{SD}$ years old) and 29 pairs of same-sex dizygotic twins (DZ; 28 men/30 women; $23.8 \pm 1.9\text{SD}$ years old) with 105-gradient direction HARDI at 4 Tesla. We used the TDF to fit a continuous mixture of tensors and compute a weighted FA measure at each voxel.

Results: By fitting quantitative genetic models voxelwise to DTI- and TDF-derived measures, we found several measures of connectivity were highly heritable. Among the most highly heritable were projections of the tensor orientation distribution - a probability density function derived from the TDF that encodes the angular density of fiber orientation at each voxel. Genetic effects on white matter structure were detected most powerfully when diffusion measures were projected orthogonal to the local dominant fiber orientation.

Discussion: These HARDI measures may expedite the search for genes that affect white matter integrity and connectivity.

Disclosure: A. Leow: None.

145. Overlapping Differences in Anatomical and Functional Networks in Schizophrenia

Kelvin Lim*, Brent Nelson, Camchong Jazmin, Bell Christopher, Ryan Muetzel, Bryon Mueller

University of Minnesota, Minneapolis, MN

Background: Abnormal connectivity has been proposed as a potential pathophysiological process in schizophrenia. Abnormal brain connectivity has been assessed with different methods including EEG, MEG and PET. More recent methods for measuring connectivity have included diffusion weighted tractography to examine the anatomical connections and resting state fMRI to examine functional connectivity between brain regions. Network-based statistic (NBS) has been developed by Zalesky et al (Neuroimage 2010) as an approach to identify network components that differ between groups. We applied NBS to resting fMRI data and diffusion weighted data from patients with schizophrenia and healthy comparison subjects to examine the differences in their functional and anatomical networks and to identify overlap between the components found to be different between groups.

Methods: 29 chronic schizophrenia patients (11 females, age: mean = 41.3, SD = 9.28) and 29 controls (11 females, age: mean = 41.1, SD = 10.6) were recruited. Participants underwent a 6-min resting-fMRI scan and were instructed to be as still as possible, keep their eyes closed, and stay awake (3.0T Siemens TIM Trio scanner, single shot EPI acquisition with TR = 2000 ms, TE = 30 ms, Flipangle = 90 deg FOV = 220 mm, 3.4 mm x 3.4 mm, slice thickness 4 mm with a total of 34 slices; 180 volumes over 6 minutes). DTI data were acquired axially using a dual spin echo, single-shot, pulsed gradient EPI sequence along 30 noncollinear directions. A high-resolution T1-weighted anatomical image was acquired using a magnetization prepared rapid gradient-echo sequence. FMRI preprocessing steps (FEAT-FSL) included first 3 volumes deleted to account for magnetization stabilization; motion correction; Bo fieldmap unwarping to

correct for geometric distortion using acquired field maps; slice-timing correction; non-brain removal; spatial smoothing; grand-mean and intensity normalization; highpass temporal filtering; prewhitening; registration to standard space (Montreal Neurological Institute-152 brain). Average timecourses were extracted from 90 anatomical regions of interest (nodes) defined by the AAL atlas (automated anatomical labeling, Tzourio-Mazoyer 2002). A wavelet transform was computed for each timecourse. For the frequency range .06-.125 Hz, a 90x90 matrix was created with the correlation coefficient between intersecting timecourses in each cell. DTI preprocessing steps (FSL) included eddy current correction, head motion correction and geometric distortion correction. The 90 AAL (same as fMRI) anatomical regions/nodes were transformed to the native space of each subject. Streamlines were calculated with FACT-based algorithm from TrackVis Diffusion Toolkit (trackvis.org). Custom software was written to calculate the number of streamlines connecting each of the 90 AAL nodes. Nodal degree (number of connections that link a node to another) was computed to create a 90x90 matrix. Network-based statistic was used to identify any component with pairwise associations that were significantly different between groups. Suprathresholds were selected to match number of nodes between anatomical and functional networks. Common nodes between anatomical and functional components were identified.

Results: Using a suprathreshold of $t = 5.25$ for the rsfMRI, a component consisting of 35 nodes and 53 edges was significant at $p = 0.0001$. The anatomical regions included frontal, temporal, parietal, and occipital. Using a suprathreshold of $t = 2.15$ for the DTI, a component consisting of 30 nodes and 30 edges was significant at $p = 0.006$. The anatomical regions included frontal, temporal, parietal, occipital, caudate, putamen, and thalamus. Regions that overlapped between the functional and anatomical network components included: temporal, parietal, and occipital.

Discussion: Using NBS, we were able to identify a network component difference between patients with schizophrenia and controls, in both functional and anatomical datasets. The functional network component contained many of the same regions described by Zalesky et al. The anatomical network component overlapped with 12/35 nodes identified in the functional network component suggesting that dysfunction of some network nodes is present in both the functional and anatomical networks. The extent to which this coupling is related to clinical phenotype will require the development of new analytic strategies.

Disclosure: K. Lim: None. B. Nelson: None. C. Jazmin: None. B. Christopher: None. R. Muetzel: None. B. Mueller: None.

146. Cerebellar Activation on a Finger-Tapping Task is Associated with Cerebellar Volume in Healthy Youths but not in Youths with Attention-Deficit/Hyperactivity Disorder

Melissa Lopez-Larson*, Jadwiga Rogowska, Deborah Yurgelun-Todd

University of Utah, Salt Lake City, UT

Background: Youths with attention-deficit/hyperactivity disorder (ADHD) have motor abnormalities including slow performance, greater variability in motor speed and greater degree of motor overflow. One neuroimaging study examined brain activation in youths with ADHD compared to healthy controls (HC) on an alternating finger tapping task and found reduced activation in BA 4 (primary motor cortex) in ADHD youths (Mostofsky 2006). This study focused its ROI analysis to BA 4 and did not report cerebellar activation. However cerebellar abnormalities have been reported on recent fMRI studies during cognitive tasks and reduced cerebellar volumes have consistently been reported in ADHD (Cherkasova 2009). To determine if cerebellar activation abnormalities and cerebellar volume abnormalities co-presented in ADHD youths we acquired high resolution morphometric data and fMRI data during a bilateral finger-tapping sequence in a group of ADHD youths compared to HC. We hypothesized that reduced cerebellar activations would be present in youths with ADHD compared to HC and that this reduction in activation within the ADHD group would be positively associated with cerebellar volumes.

Methods: Nine youths with ADHD (aged 13.7 ± 3.3), unmedicated for at least 36 hours, and 9 HC (aged 13.6 ± 3.2), matched for age and gender,

underwent psychiatric evaluation including the KSADS-PL and an MRI scan on a 3T magnet using a 12-channel head coil. Both a high-resolution structural scan (3D MPRAGE grappa sequence acquired sagitally) and an fMRI scan were obtained, which included a bilateral self-paced finger tapping sequence in a block design. Cortical reconstruction and volumetric segmentation was performed to obtain cerebellar volumes utilizing the Freesurfer image analysis suite, which is documented and freely available for download online (<http://surfer.nmr.mgh.harvard.edu/>). Functional MRI data was analyzed using general linear model and the SPM5 software package in Matlab. Region of interest (ROI) analyses were performed between groups for Brodmann's areas 4 (motor cortex) and the cerebellum. Linear regressions were performed for cerebellar activation and cerebellar volume for both groups.

Results: Two participants with ADHD were removed from the analyses secondary to motion. The mean total cerebellar volumes were not statistically significant between groups ($HC = 117.7 \pm 7.1 \text{ cm}^3$ and $ADHD = 112.3 \pm 6.3 \text{ cm}^3$). For ROI analysis, youths with ADHD had reduced activation in BA4 ($T_{\max} = 2.75$, $k = 21$, $x = -16$, $y = -24$, $z = 63$) and cerebellum ($T_{\max} = 3.06$, $k = 670$, $x = -7$, $y = -62$, $z = -23$). A significant negative regression was found between cerebellar activation and cerebellar volume in HC's only ($T_{\max} = 11.39$, $k = 54$, $x = 14$, $y = -56$, $z = -36$).

Discussion: We found ADHD youths had reduced cerebellar activation compared to HC. This finding is congruent with recent studies that have found abnormal activation in the cerebellum on cognitive tasks². Although not statistically significant in this small sample, ADHD youths also had smaller overall cerebellar volumes. In healthy controls, a reduction in cerebellar volume was associated with an increase in activation in the cerebellar region. This association was not found in youths with ADHD. These preliminary findings suggest that a reduction in cerebellar volume in ADHD may not have the normative compensatory increase in cerebellar activation that is seen in HC. Further multimodal neuroimaging investigations of the role of the cerebellum in the pathophysiology of ADHD are warranted. 1. Mostofsky SH, Rimrodt SL, Schafer JG, Boyce A, Goldberg MC, Pekar JJ, Denckla MB. Atypical motor and sensory cortex activation in attention-deficit/hyperactivity disorder: a functional magnetic resonance imaging study of simple sequential finger tapping. *Biol Psychiatry*. 2006;59:48-56. 2. Cherkasova MV, Hechtman L. Neuroimaging in attention-deficit hyperactivity disorder: beyond the fronto-striatal circuitry. *Can J Psychiatry*. 2009;54:651-64.

Disclosure: M. Lopez-Larson: None. J. Rogowska: None. D. Yurgelun-Todd: Part 1; Consultant to Kyowa Hakko, Eli Lilly, Janssen.

147. Orbitofrontal Cortex and Drug Use During Adolescence: Role of Prenatal Exposure to Maternal Smoking and Epigenetic Modifications of the Brain Derived Neurotrophic Factor Gene

Shahrdad Lotfipour*, Maria Toledo-Rodriguez, Eamonn Ferguson, Gabriel Leonard, Michel Perron, Bruce Pike, Louis Richer, Jean SéGuin, Roberto Toro, Suzanne Veillette, Zdenka Pausova, Tomas Paus

Rotman Research Institute, University of Toronto, Toronto, Ontario, Canada, CEGEP Jonquiere, Jonquiere, Quebec, Canada, Department of Psychology, University of Quebec in Chicoutimi, Chicoutimi, Quebec, Canada, Department of Psychiatry, Université de Montréal, Montréal, Quebec, Canada, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada, School of Psychology, University of Nottingham, United Kingdom, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, University of California-Los Angeles, Los Angeles, CA

Background: Despite persistent public health initiatives, nearly 17% of pregnant women continue to smoke during pregnancy in the United States. Prenatal exposure to maternal cigarette smoking

(PEMCS) is known to have long-term consequences on the physical and mental health of the offspring, including obesity, experimentation with drugs, and attention-deficit hyperactivity disorder. It has been hypothesized that increased substance use behavior may be mediated by changes in the function and/or structure of the orbitofrontal cortex (OFC). In this study, we tested this hypothesis by evaluating the relationship between OFC thickness and drug experimentation in adolescents, half of whom had PEMCS. We predicted that genes involved in brain development and epigenetic mechanisms could mediate structural modifications of the OFC, particularly associated with the brain derived neurotrophic factor (BDNF) gene.

Methods: The behavioral data set included 597 adolescents (275 sibships; 12-18 years of age). Analysis of cortical thickness and genotyping was performed using available data from 314 adolescents. The likelihood of substance use was assessed with the Diagnostic Interview Schedule for Children Predictive Scales. The number of different drugs tried by each adolescent was assessed using a separate questionnaire. Thickness of the OFC was estimated from T1-weighted magnetic resonance images using FreeSurfer software. Based on PEMCS and the level of drug experimentation, 156 adolescents (valine/valine carriers) were selected for BDNF methylation analysis. Analysis of DNA methylation was performed through bisulfite sequencing.

Results: Our findings show that: (1) PEMCS is associated with the thinning of the orbitofrontal cortex and that (2) this thinning in exposed individuals correlates negatively with drug experimentation during adolescence. We propose that these relationships could be mediated by exposure-related down regulation of genes involved in brain development through epigenetic mechanisms. Furthermore, in non-exposed adolescents, we observe a clear effect of BDNF genotype (valine/valine homozygotes vs. methionine carriers) on the relationship between cortical thickness and drug experimentation; an effect that is absent in the adolescents exposed to maternal cigarette smoking. These long-term effects may be mediated by epigenetic changes in the human genome. We speculate that the absence of the BDNF-genotype effect in the exposed adolescents might be due to an increased methylation of cytosine-phosphodiester-guanosine (CpG) islands in the promoter regions of this gene, thus down-regulating expression of the gene in both valine/valine homozygotes and methionine carriers. Our follow up studies evaluate two regulatory regions in the BDNF gene: (1) the BDNF-4 promoter, previously shown in animal studies to decrease BDNF expression after DNA methylation; and (2) the BDNF-6 promoter and 5'-untranslated region, which is located on a CpG island with one of the highest densities of CpGs (53) in the BDNF gene. Results demonstrate that PEMCS is associated with greater methylation of the BDNF-6 promoter and 5'-untranslated regions, but not of the BDNF-4 promoter region. Compared with non-exposed adolescents, adolescents exposed prenatally to maternal cigarette smoking had nearly a fourfold higher rate of methylation in the BDNF-6 promoter and 5'-untranslated region.

Discussion: We speculate that PEMCS interferes with the development of the OFC through enhanced DNA methylation of the BDNF gene and, in turn, may influence the likelihood of drug use among adolescents. In contrast, we suggest that, among non-exposed adolescents, drug experimentation influences the OFC thickness via processes akin to experience-induced plasticity. In conclusion, our findings highlight the consequences of PEMCS on the brain and behavior of adolescents and suggest that epigenetic modifications of the BDNF gene may modify, at least in part, these effects.

Disclosure: S. Lotfipour: Part 1; Pfizer. Part 4; Pfizer. M. Toledo-Rodriguez: None. E. Ferguson: None. G. Leonard: None. M. Perron: None. B. Pike: None. L. Richer: None. J. Séguin: None. R. Toro: None. S. Veillette: None. Z. Pausova: None. T. Paus: None.

148. Fronto-Temporal Anatomical Connectivity and Suicidality in Bipolar Disorder: A Diffusion Tensor Imaging Study
 Katie Mahon*, Katherine Burdick, Jinghui Wu, Toshikazu Ikuta, Babak Ardekani, Philip Szeszko

The Feinstein Institute for Medical Research, Manhasset, NY

Background: Patients with bipolar disorder have a high rate of suicide attempts and completion. Abnormalities in fronto-temporal connectivity are hypothesized to play an important role in mood dysregulation and thus, may have strong relevance for suicide risk. The uncinate fasciculus, a large white matter tract that connects the temporal and orbitofrontal cortices, is of particular interest in clarifying the neuroanatomical mechanisms that may underlie bipolar disorder and associated risk for suicide. In this study we examined the uncinate fasciculi in patients with bipolar disorder with and without a documented history of suicide attempts.

Methods: Diffusion tensor imaging exams were acquired in 14 patients with bipolar disorder with a history of suicide attempts, 15 patients with bipolar disorder without an attempt history and 15 healthy volunteers. The Tract Based Spatial Statistics algorithm in the FSL software package was used to create a group estimate of the center of each major white matter tract, resulting in a white matter "skeleton." Masks comprising the left and right uncinate fasciculi were placed on this group skeleton. Mean fractional anisotropy (FA) within these masks was then calculated for each subject and exported to SPSS for group comparisons.

Results: Patients with bipolar disorder and a history of suicide attempts had significantly ($p < .05$) lower mean FA along the right uncinate compared to healthy volunteers. Patients without a history of suicide attempts had FA values in the right uncinate that were intermediate between patients with a history of suicide attempts and healthy volunteers. None of the groups differed significantly in mean FA along the left uncinate fasciculus.

Discussion: Patients with bipolar disorder and a history of suicide attempts demonstrated lower FA in the right, but not the left uncinate fasciculus compared to healthy volunteers. Our findings support models of bipolar disorder that implicate fronto-temporal disconnection as a possible mechanism underlying mood dysregulation and vulnerability to suicide. Moreover, lateralization of this effect is consistent with evidence that right-hemisphere dysfunction is related to the generation and maintenance of extreme mood states and may have implications for the neurobiology of suicidal behavior.

Disclosure: K. Mahon: None. K. Burdick: Part 1; Merck. J. Wu: None. T. Ikuta: None. B. Ardekani: None. P. Szeszko: Boehringer Ingelheim.

149. Cortical Beta-Amyloid Deposition in Late Life Depression

Christopher Marano*, Clifford Workman, Yun Zhou, Cynthia Munro, Daniel Holt, Constantine Lyketsos, Dean Wong, Gwenn Smith

Johns Hopkins University School of Medicine, Baltimore, MD

Background: Late-life depression (LLD) is a major public health problem that is associated with disability, mortality, and an increased rate of completed suicide. Despite the availability of effective antidepressant agents, many depressed patients are refractory to treatment. Cognitive impairment is a common feature of LLD which frequently persists even after mood symptom remission. Furthermore, substantial evidence indicates that LLD is both a risk factor and/or a prodrome of dementia including Alzheimer's disease (AD). A logical focus of molecular imaging studies is to evaluate whether persistent cognitive impairment in LLD is associated with AD neuropathology. Serial positron emission tomography (PET) imaging studies performed during the course of antidepressant treatment have shown that changes in cerebral glucose metabolism in a medial temporal-parietal-frontal network are associated with underlying improved cognition in LLD (Diaconescu et al., 2010). This network overlaps with the brain regions affected in AD, as well as the regional distribution of

beta-amyloid deposition. The present study tested the hypothesis that cortical beta-amyloid deposition was higher in LLD patients relative to controls.

Methods: Six un-medicated LLD patients (4 females/2 males, age 63.5 ± 4.6 , Mini Mental State Examination [MMSE] score 29.5 ± 1.2) who met DSM-IV criteria for major depression, but who did not meet Petersen criteria for mild cognitive impairment (MCI), and 6 non-depressed age-matched controls (2 females/3 males, age 64.6 ± 6.5 , MMSE score 29.4 ± 0.9) were enrolled in the study. All participants underwent baseline neuropsychological assessment, brain magnetic resonance (MR) imaging with a Philips 3T Achieva scanner, and PET scans with the CPS/Siemens High Resolution Research Tomograph (HRRT) scanner using the radiotracer [¹¹C]-Pittsburgh Compound B ([¹¹C]-PIB; N-methyl-[¹¹C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole) to measure beta-amyloid deposition. Then, LLD patients began citalopram treatment at 10 mg daily and were titrated to a maximal dose of 60 mg based on clinical response (mean dose 30 ± 16.7 mg). Follow-up neuropsychological testing was performed at 8-10 weeks of treatment, after achieving a stable clinical response for 2 weeks. Both hypothesis driven, volume of interest (VOI) and data driven, voxel-wise PET data analysis methods were performed (Zhou et al., 2007). Distribution volume ratio (DVR), an index of tracer specific binding to beta-amyloid, was estimated using a simplified reference tissue model (SRTM). The cerebellar VOI was used for the input function for the SRTM analysis. Voxel-wise analyses of the parametric DVR images were performed with Statistical Parametric Mapping (SPM5).

Results: In LLD patients, depressive symptoms decreased significantly as measured with the 17-item Hamilton Depression Rating Scale (HAM-D; baseline: 17.3 ± 1.2 ; follow-up: 3 ± 1.7 ; $p < 0.01$). Relative to controls, LLD patients performed worse on tests of verbal fluency, verbal and visuospatial memory (effect sizes: 1.08, 0.16, 1.31, respectively). ROI analysis showed significantly greater beta-amyloid deposition in LLD patients than controls in the anterior cingulate gyrus, superior and middle frontal gyrus and precuneus (DVR range: controls: 1.10-1.15 and LLD patients: 1.42-1.50; $p < 0.05$). Voxel-wise analyses confirmed greater beta-amyloid in these areas, as well as the bilateral insula, left orbito-frontal gyrus and left parahippocampal gyrus ($p < 0.01$).

Discussion: Greater beta-amyloid deposition in non-MCI, LLD patients was observed relative to controls in similar cortical association areas that show beta-amyloid deposition in non-depressed MCI patients. These preliminary data suggest that beta-amyloid may be associated with cognitive impairment in LLD.

Supported in part by MH 1621, MH 64823, MH 86881 and RR 25005

Disclosure: C. Marano: Part 1; Pfizer. Part 4; Pfizer. C. Workman: None. Y. Zhou: None. C. Munro: None. D. Holt: None. C. Lyketsos: Part 1; Forest, Glaxo-Smith-Kline, Eisai, Pfizer, Astra-Zeneca, Ortho-McNeil, Bristol-Myers, Novartis, Elan, Supernus, Adlyfe, Takeda, Wyeth, Lundbeck, Merz, Genentech, Health Monitor, Lilly. Part 4; Forest, Glaxo-Smith-Kline, Eisai, Pfizer, Astra-Zeneca, Ortho-McNeil, Bristol-Myers, Novartis, Elan, Lilly, Associated Jewish Federation of Baltimore, Weinberg Foundation, National Football League. D. Wong: Part 1; Acadia, Amgen, Inc, Avid, Bristol-Myers Squibb, Eli Lilly, Intracellular, Johnson & Johnson, Merck, Orexigen, Roche, Sanofi-Aventis. Part 4; Acadia, Amgen, Inc, Avid, Bristol-Myers Squibb, Eli Lilly, Intracellular, Johnson & Johnson, Merck, Orexigen, Roche, Sanofi-Aventis. G. Smith: Part 1; Pfizer. Part 4; Pfizer.

150. Genetic Association Of Erbb4 And Gaba Levels In Vivo

Stefano Marenco*, Matthew Geramita, Jan Willem Van der Veen, Christine Rebsch, Antonina Savostyanova, Alan S. Barnett, Bhaskar Kolachana, Jun Shen, Daniel Weinberger, Amanda J. Law

NIMH/GCAP, Bethesda, MD

Background: Genetic and post-mortem studies have implicated Erbb4 in the pathogenesis of schizophrenia, however the biological mechanisms remain unclear. Recent work suggests that Erbb4 is expressed predominantly on inhibitory interneurons (Fazzari et al., 2010) and that

its presence on parvalbumin positive interneurons mediates the effects of neuregulin 1 (an independent risk gene for schizophrenia) on pyramidal cell activity. Therefore, Erbb4 plays a critical role in regulating the development and function of both excitatory input and inhibitory output synapses of parvalbumin positive interneurons. Previous work has demonstrated that a schizophrenia risk haplotype in ErbB4 (marked by rs7598440) predicts elevated expression of ErbB4 CYT-1 in the human brain (Law et al. 2007). We therefore hypothesized that genetic variation in ErbB4, via its effects on ErbB4 expression, would affect indices of GABA transmission. We tested this hypothesis in healthy volunteers by measuring GABA and GLX (a peak containing primarily glutamate and glutamine) with magnetic resonance spectroscopy (MRS). We hypothesized that the risk allele at rs7598440 would be associated with increased GABA levels because of its prior association with elevated ErbB4 CYT-1 in the post-mortem human brain.

Methods: 116 healthy volunteers were scanned at 3T with a proton MRS PRESS based j-editing sequence in a single voxel centered on the dorsal anterior cingulate gyrus straddling the two hemispheres (TR/TE 1500/68 ms, 18cc). Dependent variables were ratios of GABA/Creatine (Cre), GABA/H₂O, Cre/H₂O, GLX/Cre, and GLX/H₂O. All these variables were transformed to z-scores to account for different scanners used. Effects of ErbB4 were explored in conjunction with Age, Sex and % gray matter in the voxel as potential covariates. Statistical analysis was conducted with multiple regression using stepwise backward selection of variables and allowing all possible 2 way interactions to enter the model, while forcing the main effects of the independent variables above to be in the model.

Results: ErbB4 rs7598440 genotype significantly predicted GABA/Cre ($p = 0.008$) and GABA/H₂O ($p = 0.014$) signals with the major (risk) allele homozygotes and heterozygotes having higher GABA levels than minor allele homozygotes. The other variables indexing Cre and glutamate did not show significant main effects of ErbB4 genotype (Cre/H₂O: $p = 0.09$; GLX/Cre: $p = 0.56$; GLX/H₂O: $p = 0.51$).

Discussion: Our results demonstrate that in normal individuals the schizophrenia risk allele (A) at ErbB4 rs7598440 is associated with elevated *in-vivo* GABA levels as compared with non-risk allele homozygotes (G/G). This could be the result of increased formation of GABAergic synapses during development due to excess CYT-1 ErbB4 isoform. Indices of glutamatergic function were not affected in this study, serving as a potentially important negative finding consistent with predictions from animal studies. Future work will focus on measures of glutamate alone. References: Fazzari et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signaling. *Nature* 2010, 464(7293):1376-80. Law et al. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. *Hum Mol Genet*. 2007, 16(2):129-41.

Disclosure: S. Marenco: None. M. Geramita: None. J. van der Veen: None. C. Rebsch: None. A. Savostyanova: None. A. Barnett: None. B. Kolachana: None. J. Shen: None. D. Weinberger: None. A. Law: None.

151. Dopamine Synthesis and Resting Brain Activity in Parkinsonism Associated with Gaucher Disease

Joseph Masdeu*, Ozlem Goker-Alpan, Angela Ianni, Philip Kohn, Maureen Chalfin, Daniel Eisenberg, Grisel Lopez, Ellen Sidransky, Karen Berman

National Institutes of Health, Bethesda, MD

Background: Mutations in the gene encoding glucocerebrosidase (GBA), the enzyme deficient in Gaucher disease, are a common genetic risk factor for parkinsonism: subjects with Parkinson disease (PD) are over five times more likely to carry GBA mutations than healthy controls (Sidransky et al., 2009). The clinical phenotype of PD patients with GBA mutations resembles the phenotype of sporadic PD, except for a younger age at onset and more cognitive impairment. To evaluate the neurobiology of parkinsonism with and without GBA mutations, we measured two relevant variables by means of positron emission tomography (PET): regional brain dopamine synthesis and

regional cerebral blood flow (rCBF), an index of local neural activity, during wakeful rest.

Methods: Using standard procedures, we measured brain dopamine synthesis with ¹⁸F-fluorodopa PET and resting rCBF with H₂O PET in a total of 103 subjects (38F/65M), comprising four study groups: (1) Patients with sporadic PD [N = 8, average age = 60 ± 6 years]; (2) Gaucher patients with parkinsonism [N = 8, 54 ± 10 years]; (3) Gaucher patients without parkinsonism [N = 10, 50 ± 13 years]; and (4) healthy Gaucher carriers [N = 7, 50 ± 18 years]. Subjects in the last two groups had a family history of parkinsonism. Because onset of parkinsonism is earlier in GBA mutation carriers, we compared each of the four study groups to its own healthy control group, which was specially matched for age and sex. Each of the four non-overlapping control groups contained approximately twice the number of subjects than their respective patient group. Data were assessed with both region of interest and voxel-based methods.

Results: Disease duration and Unified Parkinson Disease Rating Scale scores were similar in the two groups of parkinsonian patients. Striatal dopamine synthesis was similarly decreased in both sporadic PD and GBA associated parkinsonism, and both groups had the greatest loss in the caudal striatum (putamen Ki loss: 43% and 49 respectively), with less reduction in the caudate (24% and 27% loss). Striatal dopamine was also slightly decreased ($p < 0.05$) in the putamen of Gaucher patients without parkinsonism, with the effect being significant (voxel-based, $p < 0.001$ uncorrected) in two subjects. Resting rCBF was decreased only in the Gaucher patients with parkinsonism, in a pattern also observed in diffuse Lewy body disease, involving the lateral parieto-temporo-occipital association cortex and precuneus bilaterally.

Discussion: Our study demonstrates that patients with Gaucher and parkinsonism have a pattern of dopamine loss similar to sporadic PD. Had the ventral tegmental area (VTA) been more affected than in sporadic PD, we would have observed a greater dopamine loss in its projection region, namely head of the caudate nucleus and ventral striatum. Thus, the greater cognitive impairment reported in GBA parkinsonism is not likely related to damage of the VTA, the portion of the midbrain dopaminergic system most involved in cognition and which is spared in sporadic PD (Phani, et al., 2010). By contrast, patients with Gaucher and parkinsonism tend to have decreased resting activity in brain areas similar to patients with diffuse Lewy body disease or other neurodegenerative disorders associated with cognitive impairment, such as Alzheimer's disease (Firbank et al., 2003; Silbert and Kaye, 2010). This finding may help explain the greater incidence of cognitive impairment reported in PD patients carrying GBA mutations (Goker-Alpan et al., 2008; Sidransky et al., 2009). References: Firbank MJ, Colloby SJ, Burn DJ, McKeith IG, O'Brien JT (2003) Regional cerebral blood flow in Parkinson's disease with and without dementia. *Neuroimage* 20:1309-1319. Goker-Alpan O, Lopez G, Vithayathil J, Davis J, Hallett M, Sidransky E (2008) The spectrum of parkinsonian manifestations associated with glucocerebrosidase mutations. *Arch Neurol* 65:1353-1357. Phani S, Gonye G, Iacovitti L (2010). VTA neurons show a potentially protective transcriptional response to MPTP. *Brain Res* 1343:1-13. Sidransky E et al. (2009) Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med* 361:1651-1661. Silbert LC, Kaye J (2010) Neuroimaging and cognition in Parkinson's disease dementia. *Brain Pathol* 20:646-653.

Disclosure: J. Masdeu: None. O. Goker-Alpan: None. A. Ianni: None. P. Kohn: None. M. Chalfin: None. D. Eisenberg: None. G. Lopez: None. E. Sidransky: None. K. Berman: None.

152. Accelerated Age-Related Decline in fMRI Brain Activation From Adolescence to Adulthood in the Schizophrenia Prodrome

Daniel Mathalon*, Shefali Brahmbhatt, Kasper Jorgensen, Kent Kiehl, Vince Calhoun, Thomas McGlashan, Godfrey Pearlson, Scott Woods

University of California, San Francisco, San Francisco, CA

Background: Brain abnormalities evident in schizophrenia are generally thought to arise from faulty neurodevelopment, but the

timing of these putative developmental abnormalities remains to be elucidated. While early neurodevelopmental insults have been implicated in the pathogenesis of schizophrenia (e.g., increased risk of schizophrenia in the offspring of women who are pregnant during viral epidemics or who experience obstetric complications), abnormal brain development during adolescence is of particular interest because schizophrenia symptoms typically arise during this developmental period. While it is possible that the normal neuromaturational changes (e.g., synaptic strengthening, pruning, axonal myelination) during adolescence[1, 2] unmask the illness in vulnerable individuals, several theorists have posited that abnormal brain maturation (e.g., over-pruning of synaptic connections) during adolescence underlies the progression to full-blown schizophrenia [3, 4] and may coincide with the emergence of attenuated psychotic symptoms and disruptions in daily functioning that characterize the prodromal period preceding the onset of psychosis. However, few studies have directly compared the trajectories of brain development between typically developing individuals and those at risk for schizophrenia. Accordingly, we compared age-related changes in fMRI brain activity from early adolescence to early adulthood in healthy individuals and patients at ultra-high risk for psychosis during performance of an auditory oddball task.

Methods: fMRI activity associated with target detection during an auditory oddball task was obtained from 90 healthy controls (HC) and 33 patients at ultra-high risk (UHR) for psychosis. Normal aging effects on target-related activity were modeled in the HC group using linear regression, yielding age-specific predicted brain activations. Residuals from these predicted values were generated for both groups and divided by the standard error of regression, yielding normal maturation-adjusted z-score maps. These z-score maps were compared in UHR and HC groups. Further, age-regressions were performed on these maps in UHR patients to detect abnormal maturational changes from adolescence to adulthood.

Results: UHR patients showed reduced target-related activity relative to HC in cingulate gyrus, right superior frontal gyrus, and left medial frontal gyrus ($p < .01$, uncorrected). Additionally, two clusters within bilateral temporal cortex showed negative correlations with age in UHR patients ($p < .05$, corrected), an effect that differed significantly from the absent age relationship evident in an age-matched subsample of HC subjects ($p < .05$, corrected).

Discussion: Bilateral temporal cortex showed an accelerated age-related decline, over and above normal maturational changes, in UHR patients from early adolescence to early adulthood, consistent with an altered trajectory of functional brain development during adolescence associated with risk for impending psychosis. References 1. Huttenlocher, P.R., Synaptic density in human frontal cortex: Developmental changes and effects of aging. *Brain Research*, 1979. 163: p. 195-205. 2. Yakovlev, P.I. and A.-R. Lecours, The myelogenetic cycles of regional maturation of the brain, in *Regional Development of the Brain in Early Life*, A. Minkowski, Editor. 1967, Blackwell Scientific Publications: Oxford. p. 3-70.

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Disclosure: D. Mathalon: Part 1; Pfizer. Part 4; AstraZeneca. S. Brahmabhatt: None. K. Jorgensen: None. K. Kiehl: None. V. Calhoun: None. T. McGlashan: None. G. Pearlson: None. S. Woods: Pfizer.

153. Proton Magnetic Resonance Spectroscopy Measurement of Brain Glutathione Supports Increased Oxidative Stress in Major Depressive Disorder

Sanjay Mathew*, James Murrough, Xiangling Mao, Sarah Pillemeyer, Dikoma Shungu

Baylor College of Medicine, Houston, TX

Background: There is increasing evidence that oxidative stress plays an important pathophysiological role in severe psychiatric disorders.

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine and glycine, and serves as major intracellular antioxidant and redox regulator protecting cells against oxidative stress. Dysregulation of the GSH system has been hypothesized to reduce glutamatergic activity at the NMDA receptor and attenuate neurotrophin production, processes functionally linked to cognitive and affective symptoms in major depressive disorder (MDD). While previous ¹H MRS studies in MDD have identified regional brain abnormalities in the amino acid neurotransmitters GABA and glutamate + glutamine (Glx), no study to our knowledge has measured *in vivo* brain GSH in MDD. In this pilot study, we aimed to test the hypothesis that oxidative stress is central to the pathophysiology of MDD and that GSH levels would accordingly be decreased.

Methods: Ten patients (6 females, mean age = 33.5 ± 10.7) met the diagnosis of MDD by DSM-IV-TR criteria and confirmed by SCID interview. Psychiatric conditions commonly comorbid with MDD (e.g. substance abuse or dependence) were excluded. MDD patients had moderate-to-severe depressive symptoms (QIDS-SR = 16.7 ± 4.8) at the time of scan. To avoid confounds of psychotropic medication usage on neuroimaging measures, all subjects were psychotropic medication-free for at least 2 weeks prior to scanning. Twelve non-psychiatrically ill, medically healthy volunteers (6 females, mean age = 26 ± 2.5) assessed by the SCID-IV-NP, were comparison subjects. All *in vivo* brain GSH spectra were recorded from a single 3x3x2-cm³ occipital lobe voxel on a GE 3.0 T "EXCITE" MR system using the standard J-edited spin echo difference method and an 8-channel phased-array head coil. Briefly, volume-selective J-editing detection of GSH was accomplished by incorporating into the standard PRESS sequence a pair of frequency-selective "editing" pulses before and after the second 1800 rf pulse flanked by spoiler gradients of opposite signs. Each frequency-selective editing pulse was applied at 4.56 ppm (the frequency of the GSH cysteinyl α protons) on alternate scans with TE/TR 68/1500 ms, resulting in alternated inversion of the GSH cysteinyl β doublet at 2.9 ppm by alternatively inhibiting and allowing its J-modulation. Subtracting two subspectra thus acquired in 15 min with 240 interleaved excitations yielded the desired GSH resonance at 2.9 ppm, while the much stronger overlapping tCr resonance – a singlet that is not J-modulated – is eliminated. GSH peak areas were derived by frequency-domain spectral fitting and expressed as ratios relative to the area of a simultaneously acquired unsuppressed voxel tissue water (W).

Results: Mean GSH/W in the occipital cortex was significantly reduced in the MDD group compared to healthy volunteers ($P = 0.009$), representing a 23% mean reduction. Notably, 9 of 10 MDD patients had occipital GSH/W below the group mean of the healthy group, compared to 1 of 12 patients in the healthy group for the converse (Fisher Exact Test $p < 0.05$). There was a significant difference between groups in mean age ($p = 0.02$), and a negative correlation between GSH/W and age ($r = -0.622$, $p = 0.01$). However, GSH/W in the MDD group remained significantly lower than in the healthy volunteer group after adjusting for age ($p = 0.014$).

Discussion: This is the first study to our knowledge to investigate *in vivo* cortical GSH in patients with MDD. Our preliminary finding of decreased cortical GSH in MDD is consistent with pathophysiological models postulating a role for oxidative stress and oxidative metabolic pathways in mood disorders. The potential therapeutic relevance of these findings is suggested by emerging data showing that the GSH precursor N-acetyl cysteine (NAC) has benefit in mood disorders such as bipolar disorder. These preliminary results suggest further investigations in larger samples to assess antioxidant capacity in additional brain regions implicated in mood regulation. In addition, scrutiny of clinical features such as anxiety and disease chronicity, and lifestyle factors that impact GSH and oxidative stress pathways, such as physical exercise, diet, and smoking history, is warranted.

Disclosure: S. Mathew: Part 1; AstraZeneca, Pfizer, Merck, Cephalon. Part 2; AstraZeneca. Part 3; AstraZeneca. Part 4; GlaxoSmithKline, Roche, Evotec. J. Murrough: Evotec. X. Mao: None. S. Pillemeyer: None. D. Shungu: None.

154. Tolcapone Modulates Functional Coupling of the Prefrontal Cortex During Working Memory

Venkata Mattay*, José A. Apud, Roberta Rasetti, Carmela S. Magalona, Qiang Chen, Fabio Sambataro, Xi Cheng, Heather Decot, Joseph Callicott, Daniel Weinberger

NIMH, Bethesda, MD

Background: Schizophrenia has been characterized as a syndrome of altered functional coupling within cortical circuits. Consistent with this, recent studies have shown abnormal connectivity between the dorsolateral prefrontal cortex (DLPFC) and other brain regions underlying working memory in patients with schizophrenia (PTs) compared to healthy volunteers (HVs) (Esslinger et al, 2009). Evidence also indicates dopaminergic drugs modulate neural circuits including those underlying working memory (Apud et al, 2007). In the current study, we explore the effect of tolcapone, a drug that crosses the blood-brain barrier and increases dopamine levels via inhibition of the catecholamine-O-methyltransferase (COMT) enzyme, on connectivity of brain regions underlying working memory in patients with schizophrenia and healthy volunteers.

Methods: We performed a double-blinded, placebo-controlled trial with tolcapone (dose - 100 mg three times a day on the first day and 200 mg three times a day for the next 6 days) following a within-measures, counter-balanced study design. The second arm of the study was performed after a one-week wash-out period following the first arm. Thirty HV (16 males, 14 females; mean age = 31 years) and 21 PTs (15 males, 6 females; mean age = 27.2 years) underwent BOLD fMRI (3T) while performing the 2-back working memory task. Both groups were matched for age, gender, IQ (WRAT), and 2-back performance. Analysis using psycho-physiological interaction (PPI) was performed to investigate the coupling between the right DLPFC (rDLPFC), a region showing abnormal functional coupling in PTs (Esslinger et al, 2009), and other brain regions involved in working memory.

Results: PPI analyses showed positive task-load related modulation (from 0 to 2-Back) between the right DLPFC and contralateral ventrolateral prefrontal cortex (VLPFC), bilateral angular gyrus (BA39), bilateral superior frontal gyrus (BA 8, BA9), medial frontal gyrus (BA8, BA 9), and left postcentral gyrus in both HVs and PTs. A negative modulation of the task load was observed in the coupling of right DLPFC with posterior cingulate, cuneus and precuneus. Task load positively modulated the right DLPFC-hippocampus coupling in HVs, while in PTs this modulation was negative ($pFDR\text{-corr} = 0.05$). There was an effect of diagnosis with decreased positive coupling between right DLPFC and left VLPFC in PTs when compared to HVs ($xyz = -57 \ 21 \ 6$; $k = 5$, $t = 3.87$, $p < 0.001$ uncorrected). Further, task load positively modulated the right DLPFC-hippocampus coupling in HVs, while in PTs this modulation was negative ($xyz = -33 \ -33 \ -9$; $k = 3$, $t = 3.48$, $p = 0.001$ uncorrected). There was an effect of drug particularly in PTs with Tolcapone enhancing the coupling between the right DLPFC and hippocampus when compared to placebo ($xyz = -33 \ -27 \ -9$; $k = 66$, $t = 3.18$, $pFWE\text{-corr} = 0.044$). In PTs only, coupling between the right DLPFC and hippocampus shifted from negative during placebo to positive during tolcapone, mimicking the positive coupling observed in HVs. This drug effect was also observed in the enhanced coupling between right DLPFC and left VLPFC in PTs only ($xyz = -36 \ 33 \ -6$; $k = 125$, $t = 3.64$, $pFWE\text{-corr} = 0.043$).

Discussion: The results suggest that tolcapone not only modulates DLPFC activity during working memory as previously reported (Apud et al 2007), but also modulates the functional coupling of the right DLPFC with other brain regions including the VLPFC and hippocampus. Interestingly, the connectivity modulating effects of tolcapone between the right DLPFC and VLPFC, and additionally between the right DLPFC and hippocampus, were much more predominant in PTs. Overall, these results indicate that a cortically selective dopaminergic drug differentially modulates the neural circuitry underlying working memory in patients with schizophrenia compared to healthy volunteers.

Disclosure: V. Mattay: None. J. Apud: None. R. Rasetti: None. C. Magalona: None. Q. Chen: None. F. Sambataro: None. X. Cheng: None. H. Decot: None. J. Callicott: None. D. Weinberger: None.

155. Association between Marijuana and Amygdalar Volumes in Adolescents

Erin McGlade*, Melissa Lopez-Larson, Deborah Yurgelun-Todd

University of Utah Brain Institute, Salt Lake City, UT

Background: Marijuana (MJ) is the most commonly used illicit drug in the United States according to recent estimates (Substance Abuse and Mental Health Services Administration, 2009). MJ use has been related to mood disorders (SAMHSA) and to mood alterations in chronic MJ users (Degenhardt 2003), however the relationship between mood and structural brain changes in MJ abusing populations remains to be clarified. Previous investigations have reported amygdalar volume reductions in both depressed individuals (Kronenberg 2009 and Rosso 2005) and in adults with a history of heavy MJ use (Yücel 2008). To date there is a paucity of studies investigating the association between amygdalar volume, mood changes, and MJ use in adolescents. This study examined the association between amygdalar volume and depressive mood in MJ-abusing adolescents compared to healthy controls (HC) to assess whether there are differences in self-reported measures of depression and amygdalar volumes.

Methods: Eighteen adolescents with current chronic heavy MJ use and no other current Axis I disorder (aged 17.8 ± 0.9 years) and 18 age-matched HC (aged 17.3 ± 0.8 years) received MRI scans on a 3T Siemens Trio scanner. The imaging protocol included a T1-weighted 3D MPRAGE grappa sequence acquired sagitally using a 12-channel head coil (TE/TR/TI = 3.37 ms/2.0 s/1.1 s, 8° flip, 256x256 acquisition matrix, 256 mm² FOV, 160 slices, 1.0 mm slice thickness). Volumetric segmentation was performed with Freesurfer and amygdalar volumes were corrected for total brain volume. Subjects completed a structured diagnostic interview (KSADS or SCID depending on age) and Hamilton Rating Scale for Depression (HAM-D).

Results: Between group analyses found that MJ users reported significantly higher levels of depressive symptoms than HCs ($p = 0.02$) and that depressive symptoms for MJ users were related to earlier age of onset for MJ use ($p = 0.02$). Compared to HCs, MJ users also showed a trend toward lower right amygdalar volume ($p = 0.076$) and a negative association between depressive symptoms and right amygdalar volume ($p = 0.066$). No significant association was seen between lifetime MJ use and amygdalar volume. Healthy controls evidenced no correlation between amygdalar volume and measures of depressive mood.

Discussion: The findings of the current study suggest that reduced right amygdalar volume is associated with MJ use and mood state in MJ-using adolescents. Specifically, a number of depressive symptoms were significantly correlated with age of onset of MJ use and showed a trend association with right amygdalar volume in these individuals. Given the observed relationship between mood state and early onset of MJ use it is possible that increased depressive traits may influence the initiation of MJ use. Moreover, insofar as the amygdala moderates affective regulation, reduced amygdalar volume may be a predisposing risk factor for MJ use. Further investigations into the impact of depressive symptoms and amygdalar volume in association with MJ use will need to be performed. References: 1. Degenhardt, L., Hall, W., & Lynskey, M. (2003). Exploring the association between cannabis use and depression. *Addiction*, 98(11), 1493-1504. 2. Kronenberg, G., Ludger, T., Regen, F., Deuschle, M., Heuser, I., & Colla, M. (2009). Reduced amygdala volume in newly admitted psychiatric in-patients with unipolar major depression. *Journal of Psychiatric Research*, 43(13), 1112-1117. 3. Rosso, I., Cintron, C., Steingard, R., Renshaw, P., Young, A., & Yurgelun-Todd, D. (2005). Amygdala and Hippocampus Volumes in Pediatric Major Depression. *Biological Psychiatry*, 57(1), 21-26. 4. Yücel, M., Solowij, N., Respondek, C., Fornito, A., Lubman, D.,

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Disclosure: E. McGlade: None. M. Lopez-Larson: None. D. Yurgelun-Todd: Part 1; Consultant to Kyowa Hakko, Eli Lilly, Janssen.

156. Vascular Risk and PET of Brain Amyloid and Tau in Persons Without Dementia

David Merrill*, Prabha Siddarth, Linda Ercoli, Alison Burggren, Vladimir Kepe, Karen Miller, Jeanne Kim, Helen Lavretsky, Paul Thompson, S. C. Huang, Susan Bookheimer, Jorge Barrio, Gary Small
UCLA, Los Angeles, CA

Background: Vascular risk factors contribute to the likelihood of developing Alzheimer's disease, but how such risk factors relate to amyloid senile plaque and tau neurofibrillary tangle burden is uncertain. Positron emission tomography (PET) scans after intravenous injections of 2-(1-[6-[(2-[F-18]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)malononitrile (FDDNP) provide a measure of plaques and tangles *in vivo*. Here we investigate whether vascular risk factors are correlated with FDDNP binding levels in persons without dementia.

Methods: Design: Cross-sectional clinical study. Setting: A university research institute. Participants: Volunteer sample of 101 primarily normotensive middle-aged and older adults without dementia (mean age \pm SD, 64.4 ± 11.3 years), including 46 with normal aging and 55 with mild cognitive impairment (MCI), a risk state for Alzheimer's disease. Main Outcome Measures: Vascular risk factors of body mass index (BMI), systolic blood pressure (sBP), diastolic blood pressure (dBP), and total cholesterol; global FDDNP binding and FDDNP binding in medial temporal, lateral temporal, posterior cingulate, parietal, and frontal cortex regions of interest.

Results: Controlling for age and cognitive status (normal aging vs MCI), we found that BMI ($F(1,97) = 8.87$, $p = .004$) and dBP ($F(1,90) = 5.34$, $p = .02$) were associated with global FDDNP binding. Post-hoc regional analyses revealed that as BMI increased, FDDNP binding in the lateral temporal region increased ($r = 0.31$, $p = 0.003$) and as dBP increased, FDDNP binding in lateral temporal ($r = 0.24$, $p = 0.02$) and frontal ($r = 0.26$, $p = 0.01$) regions increased. Systolic BP and total cholesterol were not significantly associated with FDDNP binding levels.

Discussion: These findings suggest that some forms of vascular risk, in particular BMI and dBP, are correlated with the extent of cerebral amyloid and tau brain pathology in people without dementia. Such relationships might be useful in predicting neuropathological changes in the brain years before symptoms of dementia are present and in monitoring the efficacy of potential preventative measures.

Disclosure: D. Merrill: None. P. Siddarth: None. L. Ercoli: None. A. Burggren: None. V. Kepe: None. K. Miller: None. J. Kim: None. H. Lavretsky: Part 1; Eisai, Janssen, Pfizer, Forest. Part 4; Forest Research Institute. P. Thompson: None. S. Huang: Part 1; Glaxo-SmithKline, FDDNP-PET co-inventor (UCLA owns US patent). S. Bookheimer: None. J. Barrio: Nihon Medi-Physics Co, Bristol-Meyer Squibb, PETNet Pharmaceuticals, Siemens, FDDNP-PET co-inventor (UCLA owns US patent). G. Small: Abbott, Dakim, Eisai, Forest, Medivation, Novartis, and Pfizer, FDDNP-PET co-inventor (UCLA owns US patent).

157. Ph-mri Study Of Selective Adenosine A_{2A} Antagonist Syn115 In Cocaine Dependent Subjects

F Gerard Moeller*, Joel Steinberg, Stephen Bandak, Kimberly Kjome, Liangsoo Ma, Nuvan Rathnayaka, Larry Kramer, Ponnada Narayana

University of Texas Health Science Center at Houston, Houston, TX

Background: Brain imaging studies in cocaine dependent subjects have provided evidence of reduced dopamine receptor binding and

decreased dopamine release after doses of stimulants. Likewise, there is some preliminary evidence that medications that enhance dopamine can reduce cocaine use in humans. One novel class of medications that is being studied for Parkinson's disease due to its ability to enhance dopamine function is the selective adenosine A_{2A} receptor antagonists. As a first step in the evaluation of the selective A_{2A} antagonist SYN115 as a potential treatment medication for cocaine dependence, a Ph-MRI study was completed to determine the effects of SYN115 on brain function in cocaine dependent subjects.

Methods: 12 cocaine dependent subjects underwent two fMRI scans (one after a dose of placebo and one after a dose of 100 mg of SYN115) while performing the IMT/DMT working memory task with 3, 5, and 7 digit stimuli. Subjects were observed overnight after the dose of SYN115.

Results: SYN115 was well tolerated with no adverse events. Ph-MRI results showed that for 7-digit DMT working memory activation (relative to 7-digit IMT), SPM8 second level (Random Effects) paired t-test showed two significant clusters (false-discovery-rate corrected cluster two-tailed $p < 0.05$) in which SYN115 produced greater activation than placebo. The significantly greater activation from SYN115 occurred in bilateral (LR) anterior cingulate gyrus (g), LR superior frontal (f) g, LR superior medial g, LR middle fg, left (L) inferior fg pars triangularis, L inferior fg pars opercularis, LR medial orbital fg, L superior orbital fg, L middle orbital fg, L inferior orbital fg, L g rectus, LR supplementary motor area, L precentral g, L postcentral g, LR caudate, and L insula. Several of these regions (L caudate, L superior medial g, L middle fg, and L superior fg) overlapped with regions that had significantly less pretreatment activation in cocaine dependent subjects in a previous study (Moeller et al., 2010).

Discussion: This study provides preliminary evidence that: 1. The selective adenosine A_{2A} receptor antagonist SYN115 is well tolerated in cocaine dependent subjects. 2. SYN115 at this dose increased brain activation compared to placebo in prefrontal cortex, caudate and insula, possibly through enhancing dopamine function. 3. Several of these brain regions have been shown to have reduced function compared to normal controls. This data supports further research on SYN115 as a potential treatment for cocaine dependence.

Disclosure: F. Moeller: None. J. Steinberg: None. S. Bandak: Part 5; Synosia Therapeutics. K. Kjome: None. L. Ma: None. N. Rathnayaka: None. L. Kramer: None. P. Narayana: None.

158. Enhanced Midbrain Response Following Sustained Abstinence in Cocaine Addicted Individuals: Indications of Improvement

Scott Moeller*, Dardo Tomasi, Patricia Woicik, Thomas Maloney, Nelly Alia-Klein, Frank Telang, Gene-Jack Wang, Ruiliang Wang, Nora Volkow, Rita Goldstein

Brookhaven National Laboratory, Upton, NY

Background: Drug addiction is marked by decreased baseline (tonic) dopamine receptor availability and release. Although these drug-mediated neuroadaptations are long-lasting, positron emission tomography (PET) studies have pointed to a partial restoration of dopamine receptor availability and associated improvements in dopaminergic neurotransmission with extended abstinence. Here we tested with functional magnetic resonance imaging (fMRI) whether sustained abstinence improves functioning in dopaminergically-innervated brain regions in cocaine addiction. We expected more activation post-treatment than pre-treatment of the midbrain (location of the ventral tegmental area and substantia nigra), which is implicated in behavioral reactivity to motivationally salient stimuli. Recovery of the control network was also expected.

Methods: Fifteen individuals with cocaine use disorder, who at study time were inpatients at treatment facilities located in the greater New York area, underwent fMRI while completing an emotionally salient cognitive control task (our drug Stroop task, in which subjects press for ink color of drug vs. neutral words while receiving monetary reward for correct performance). Subjects completed this blocked

fMRI task twice: once at baseline after initial detoxification (2-4 weeks after starting treatment) and once at a 6-month follow-up (while still inpatients). During these two assessments, clinical self-report measures and objective behavioral measures of cocaine seeking/reactivity were also ascertained.

Results: Task accuracy and total money earned did not differ between baseline and follow-up, although reaction time to neutral words (but not drug words) was reduced at follow-up. Whole-brain statistical parametric mapping (SPM) analyses on the fMRI contrast follow-up > baseline revealed activations in the bilateral midbrain as expected ($p < 0.05$ cluster-level corrected). At this same statistical threshold, activations were also observed in the bilateral thalamus and right precuneus. Reduced activations (follow-up < baseline) were observed in the control network, including the pre-SMA and DLPFC (also in the bilateral fusiform gyrus and inferior parietal lobule). None of these activations differed by word type (drug vs. neutral). Importantly, whole brain correlation analyses showed that activation of the midbrain, our *a priori* region of interest, correlated with behavioral measures indicative of enhanced behavioral performance and reduced severity of addiction from baseline to follow-up. Specifically, higher midbrain activation at follow-up than baseline to the non-drug (neutral) words (compared with fixation baseline) was associated with improved task performance (higher percent correct during the neutral trials), reduced withdrawal/craving symptoms (assessed with the cocaine selective severity assessment scale), and reduced objective cocaine choice behavior (choice to view images of cocaine compared with pleasant, unpleasant, or neutral images) (for all these whole brain correlations: $p < 0.05$ cluster-corrected with a small volume correction, $r > |0.65|$, $p < 0.01$).

Discussion: Collectively, these results suggest improved dopaminergic neurotransmission following sustained abstinence in cocaine addiction. Results await comparison to a healthy control group (who also complete the task twice at baseline and 6-month follow-up) to examine whether this midbrain response normalizes. Results of these treatment-seeking subjects also await comparison to an abstinent but non-treatment-seeking group to examine if this enhanced midbrain response owes to treatment or to sustained abstinence more generally. Despite these limitations, to our knowledge the current results are the first to show elevated midbrain fMRI response following sustained abstinence, and with associated improvements in clinically-relevant outcome variables (improved cognitive performance, and reduced craving and simulated drug-seeking). Current results are relevant to the concept of allostatic in addiction, in which neuroadaptations from chronic exposure to addictive drugs produce a change in the threshold for rewards. In particular, lower severity of craving and drug seeking were paralleled by fMRI results suggesting that previously less salient stimuli (neutral words) may have become more salient following abstinence.

Disclosure: S. Moeller: None. D. Tomasi: None. P. Woicik: None. T. Maloney: None. N. Alia-Klein: None. F. Telang: None. G. Wang: None. R. Wang: None. N. Volkow: None. R. Goldstein: Part 2; Medical Directions, Inc, Federal Judicial Center, Gruter Institute for Law and Behavioral Research.

159. PH in Children and Adolescents with Bipolar Disorder

Constance M. Moore, J. Eric Jensen, Atilla Gönenç, Perry Renshaw, Jean A. King, Jean A. Frazier*

University of Massachusetts Medical School, Worcester, MA

Background: The objective of this study is to further understand the mechanisms underlying clinical symptoms of Bipolar Disorder (BD) in children and adolescents. Research exploring the correspondence between BD phenotypes and mitochondrial dysfunction, particularly in younger subjects, has been insufficient to date. However, previous studies have found abnormal cerebral pH levels in subjects with BD, which may be directly linked to abnormal mitochondrial activity.

Methods: This study utilized ^{31}P Magnetic Resonance Spectroscopy (MRS) to determine frontal lobe and global pH levels, in a group of 8 subjects diagnosed with BD and 11 healthy comparison subjects (HCS) between the ages of 8 and 20 years old.

Results: Results showed global pH was 7.00 ± 0.02 in BD subjects and 7.00 ± 0.01 in HCS. Frontal lobe pH was found to be 6.99 ± 0.03 in BD subjects ($N=7$) and 6.99 ± 0.02 in HCS ($N=8$). There were no significant difference in pH between the euthymic children with BD and the depressed/manic/mixed mood children; or between the HCS and the depressed/manic/mixed mood children. In the subjects with BD the YMRS correlated negatively with pH in the frontal lobes.

Discussion: Despite no significant difference in pH between the groups, subjects with BD had a larger range of pH values, for their age range. pH values increased with age rather than decreased in subjects with BD (contrary to other studies of HCS) perhaps a consequence of the younger subjects with BD in the study have the higher YMRS values. These results and other research suggest that over-activation of NMDA receptors and stimulation of $\text{Ca}^{2+}/\text{H}^+$ exchange within neuronal membranes may play a role in pH alterations in pediatric Bipolar Disorder.

Disclosure: C. Moore: None. J. Jensen: None. A. Gönenç: None. P. Renshaw: None. J. King: None. J. Frazier: None.

160. Exploring Gender Differences in the Neural Substrates of Stress in Cocaine Dependent Men and Women

Megan Moran-Santa Maria*, Kevin Johnson, Cullen McWhite, Colleen Reed, Kathleen Brady

Medical University of South Carolina, Charleston, SC

Background: There are significant gender differences in substance dependent individuals. For example, women appear to be more likely to relapse in response to stress than men. Interestingly, little is known about the neural correlates that underlie stress induced drug craving. The purpose of the present study was to use functional magnetic resonance imaging (fMRI) to compare brain activity between cocaine-dependent men and women during a psychosocial stressor.

Methods: Current cocaine dependence was assessed using the SCID-I/ P. Cocaine-dependent men ($n = 9$) and women ($n = 7$) were placed in a Siemens Trio 3T scanner and exposed to three runs of the Montreal Imaging Stress Task (MIST), 30 minutes after receiving an i.v. infusion of corticotropin releasing hormone. The study utilized a block design of three conditions (rest, control, and experimental). Each block lasted for 40 seconds and repeated intermittently during each of the MIST runs. During the rest period subjects were instructed to view a cross bar located on the screen and relax. During the control condition subjects were asked to perform a mental arithmetic task but told that their response would not be recorded. During the experimental condition subjects were asked to answer as quickly and accurately as possible. In addition, subjects were given immediate feedback about their performance compared with the "average performance". Subjects were given negative feedback by study personnel at the end of each run. To assess activity associated with stress, the mean activity recorded during the experimental condition was subtracted from the mean activity recorded during the control condition (experimental-control). To assess activity associated with performing the mental arithmetic task, the mean activity recorded during the rest condition was subtracted from the mean activity recorded during the control condition (control-rest). Results are based on data from sixteen subjects. Individual images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $P = 0.05$. Group images thresholded using clusters determined by corrected threshold of $P = 0.05$. Subjective ratings of stress and craving were obtained at the end of each run.

Results: There was no effect of the MIST on subjective stress or craving. The math task (control-rest) elicited activity in both cocaine dependent men and women across all three runs of the MIST. More specifically, both cocaine dependent men and women exhibited

significant activity in the prefrontal, anterior cingulate and parietal cortices in response to the math task. The stress condition (experimental-control) produced significant activity in the precuneus, anterior and posterior cingulate cortices in cocaine dependent women. In contrast, cocaine dependent men did not exhibit significant activity in response to the stress condition.

Discussion: These preliminary findings indicate that the MIST is capable of producing stress related brain activity that can be measured using fMRI. Furthermore, the MIST produces gender-specific changes in measurable brain activity related to stress, suggesting that it may be an ideal paradigm for exploring the neural correlates of stress in substance dependent individuals.

Disclosure: M. Moran-Santa Maria: None. K. Johnson: None. C. McWhite: None. C. Reed: None. K. Brady: None.

161. Cerebral White Matter Volume is Associated with Forearm Vascular Function in Elderly Men with Vascular Disease

David Moser*, Kelly Rowe, Vincent Magnotta, William Haynes, Peggy Nopoulos

University of Iowa Carver College of Medicine, Iowa City, IA

Background: Published studies, including those from our own laboratory, have revealed significant associations between forearm vascular function and cognition in individuals with atherosclerotic vascular disease (AVD). Very few studies have examined the relationship between forearm vascular function and measures of cerebral white matter volume and small vessel ischemic disease. The current analysis was conducted to determine the relationship between vascular function and cerebral white matter volume and hyperintensities in elderly men with AVD.

Methods: Participants were 17 men (Mean age = 68 years, SD = 8) with AVD, but no history of stroke, cardiac surgery, or dementia diagnosis. Vascular function was assessed in the forearm using venous occlusion plethysmography and infusion of vasoactive agents (acetylcholine, nitroprusside, verapamil), which allowed for assessment of endothelium-dependent and -independent vasodilation. Neuroimaging was conducted using a 1.5T Siemens Avanto scanner. Cerebral white matter and white matter hyperintensity volumes (both corrected for intracranial volume) were obtained using BRAINS-2 software. Total Scale Score from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used as an indicator of global cognitive function.

Results: RBANS Total Scale Score Mean = 98 (45th %ile, average range), SD = 10. When controlling for age, measures of vascular function were not significantly associated with white matter hyperintensity volume. However, controlling for age, vascular function in response to infusion of all three vasoactive agents was significantly and positively associated with cerebral white matter volume (acetylcholine: partial $r = .62$, $p = .015$; nitroprusside: partial $r = .60$, $p = .018$; verapamil: partial $r = .57$, $p = .027$).

Discussion: These preliminary findings suggest that forearm vascular function, an indicator of general vascular health, is significantly and positively associated with cerebral white matter volume in elderly men with AVD. This is intriguing, given that the participants in this study had relatively early-stage vascular disease, had no history of stroke, and performed in the average range with regard to global cognitive function. Additionally, the absence of a significant correlation between vascular function and white matter hyperintensity volume in this sample suggests the possibility that cerebral white matter volume may be a more sensitive indicator of vascular-related neuroanatomical change in this early disease stage. This study is limited by small sample size and absence of control data. Additional research is needed to determine whether vascular function is predictive of ongoing neuroanatomical and functional change in individuals with AVD.

Disclosure: D. Moser: None. K. Rowe: None. V. Magnotta: None. W. Haynes: None. P. Nopoulos: None.

162. *In Vivo* Measurement of Fluctuations in Glutamate Levels: A Positron Emission Tomography Study Using [¹¹C]ABP688 and N-Acetylcysteine

Mark Slifstein*, Nobumi Miyake, Mette Skinhjorg, Ragy Girgis, Xiaoyan Xu, Anissa Abi-Dargham

Columbia University, New York, NY

Background: *In vivo* imaging with positron emission tomography (PET) has been used to infer fluctuations in the levels of endogenous neurotransmitters following pharmacological challenge in several neurotransmitter systems. But to date, the technique has not been successfully applied to the glutamatergic system. [¹¹C]ABP688 is a PET radiotracer that is an antagonist at the mGluR₅ receptor and binds to an allosteric site on mGluR₅, suggesting it may have potential as an *in vivo* probe for glutamatergic transmission. N-acetylcysteine (NAC) has been shown to increase extracellular glutamate through stimulation of the cystine-glutamate antiporter. In this study, we used PET in anesthetized baboons to test if extracellular glutamate increase following administration of NAC could be detected as a change in [¹¹C]ABP688 binding. We compared the change in [¹¹C]ABP688 binding between baseline and NAC challenge conditions to the change between test and retest without pharmacological challenge, in order to determine if an effect of NAC could be demonstrated and shown to be robust enough to be detected above noise in the measurement.

Methods: Four adult male baboons (*papio anubis*, baboons A,B,C and D, 24 ± 4 kg) were scanned on an HR+ camera under isoflurane anesthesia. All study procedures were approved by the IACUC of Columbia University. Baboons A, B and C were scanned under test-retest conditions. Baboons A, B and D were scanned before and after 50 mg/kg NAC. Scans were performed following a single bolus of [¹¹C]ABP688. Test-retest studies were comprised of a 90 min test scan, 30 min pause and then a 90 min retest scan. NAC studies were comprised of a 90 min baseline scan, followed by a 60 min iv infusion of NAC followed by a 90 min post-challenge scan. Region of interest data were analyzed by compartment modeling with arterial plasma input. Included subcortical regions were associative striatum (AST), somatosensory-motor striatum (SMST), ventral striatum (VST), hippocampus and thalamus. Cingulate, frontal, occipital, parietal and temporal cortices were also included as well as cerebellum as a reference tissue. The main outcome measure was BP_{ND}, the binding potential with respect to nondisplaceable radioligand. The percent change between scans, Δ BP_{ND}, was compared across conditions (test-retest vs NAC) by linear mixed modeling.

Results: The mean Δ BP_{ND} \pm SD across all regions was $2 \pm 3\%$ for test-retest and $-13 \pm 7\%$ for NAC (Table 1). There was a highly significant effect of condition (test-retest vs NAC, $F(1,40) = 21.2$, $p < 0.001$).

Discussion: [¹¹C]ABP688 BP_{ND} was significantly decreased following NAC. This is the first time that changes in glutamate levels have been detected using *in vivo* imaging methods. There were no significant differences between test and retest, indicating the observed difference following NAC was not likely due to mass-carryover or some other effect of scan order. As BP_{ND} is proportional to the affinity of the radiotracer for the binding site, these results are consistent with an allosteric interaction in which the affinity of [¹¹C]ABP688 for mGluR₅ is reduced compared to baseline due to increased glutamate levels following NAC. Future studies will be required to determine if the observed effect is in fact an affinity shift and if there is an association between glutamate levels and Δ BP_{ND}. If the mechanism is confirmed, [¹¹C]ABP688 PET imaging will be a potential tool for probing glutamate transmission *in vivo*. Table 1: Δ BP_{ND} \pm standard deviation by region, p values for paired t test, n = 3 for each region and condition: AST: T-RT: $2\% \pm 11\%$, $p = 0.79$; NAC: $-12\% \pm 4\%$, $p = 0.095$ SMST: T-RT: $2\% \pm 7\%$, $p = 0.54$; NAC: $2\% \pm 18\%$, $p = 0.83$ VST: T-RT: $3\% \pm 16\%$, $p = 0.57$; NAC: $-20\% \pm 8\%$, $p = 0.094$ Cing: T-RT: $5\% \pm 14\%$, $p = 0.58$; NAC: $-16\% \pm 5\%$, $p = 0.062$ FntCtx: T-RT: $5\% \pm 20\%$, $p = 0.73$; NAC: $-16\% \pm 11\%$, $p = 0.107$ Hipp: T-RT: $-2\% \pm 14\%$, $p = 0.92$; NAC: $-14\% \pm 5\%$,

$p = 0.107$ OccCtx: T-RT: $4\% \pm 17\%$, $p = 0.82$; NAC: $-21\% \pm 13\%$, $p = 0.158$ ParCtx: T-RT: $-2\% \pm 21\%$, $p = 0.82$; NAC: $-16\% \pm 8\%$, $p = 0.121$ TemCtx: T-RT: $5\% \pm 20\%$, $p = 0.62$; NAC: $-13\% \pm 9\%$, $p = 0.167$ Thal: T-RT: $3\% \pm 11\%$, $p = 0.87$; NAC: $-6\% \pm 12\%$, $p = 0.461$.

Disclosure: M. Slifstein: Part 1; GlaxoSmithKline, Amgen Inc., intracellular Therapies, Inc. Part 4; Intra-Cellular Therapies, Inc. N. Miyake: None. M. Skinborg: None. R. Girgis: Part 1; Janssen, Lilly, Forest, Elsevier Science. Part 2; Lilly. X. Xu: None. A. Abi-Dargham: Part 1; BMS-Otsuka, Boehringer-Engleheim, Lundbeck, Sepracor, Merck. Part 2; BMS-Otsuka. Part 3; BMS-Otsuka.

163. Evidence of Age Effects in Cortical Areas But Not in the Subcortex of ADHD Children: A Multi-voxel *In Vivo* ^{31}P Spectroscopy Study at 4 Tesla

Jeffrey Stanley*, Dalal Khatib, Rachel Dick, Olivia McGarragle, Frank MacMaster, Arthur Robin, David Rosenberg

Wayne State University School of Medicine, Detroit, MI

Background: Attention-deficit/hyperactivity disorder (ADHD) is one of the most prevalent neurodevelopmental disorders in children, which is not well understood. *In vivo* ^{31}P spectroscopy is a neuroimaging method that is sensitive in detecting biochemical changes as the brain develops. Using ^{31}P spectroscopy, we have shown subcortical and cortical deficits in membrane phospholipids (MPL) precursor levels that are suggestive of an alteration of an earlier developing region influencing the maturational integration of later or slower developing regions^{1,2}. The purpose of this study is to further our investigation, using a high-field system, which dramatically improves regional specificity and biochemical resolution [e.g., the individual MPL precursors, phosphoethanolamine (PE) and phosphocholine (PC), and MPL breakdown products, glycerophospho-ethanolamine (GPE) and glycerophosphocholine (GPC)].

Methods: A total of 20 children with DSM-IV ADHD ($13\text{M} + 7\text{F}$; mean age 9.1 ± 1.4 yrs; 14 with the combined subtype and 6 with the predominantly inattentive type) and 16 healthy controls (HC; $4\text{M} + 12\text{F}$; mean age 9.3 ± 2.2 yrs) participated in this study. All ADHD children were not on any psychostimulant medication for at least a 24-hr period prior to the MR examination and no sedation was used on any subjects during the MR examination. A 3D whole-brain, multi-voxel ^{31}P spectroscopy measurement was collected in each subject on a 4 Tesla scanner using a dual-tuned $^{31}\text{P}-^1\text{H}$ head coil. The acquisition parameters of the ^{31}P spectroscopy included: FOV = $280 \times 280 \times 160$ mm 3 , phase encoding steps = $14 \times 14 \times 8$, zero-filled to $16 \times 16 \times 8$ (nominal voxel dimension = $1.75 \times 1.75 \times 2.0$ cm 3), excitation slab thickness = 80 cm (which was placed parallel to the AC-PC line to cover the whole-brain), TR = 0.54 sec, flip-angle = 33° reflecting the Ernst angle of the combined phosphocreatine (PCr), PE and PC signal, data points = 2,048, bandwidth = 4.0 kHz, 24 averages (weighted-average and elliptical k-space sampling), pre-acquisition delay time of 1.3 ms and acquisition time 23 minutes. For each bilateral region of interest (DLPFC [BA 9/46], inferior frontal gyrus [iFG; BA 44/45], dorsal anterior cingulate [dACC], striatum, and superior parietal lobe [sPL]), the $16 \times 16 \times 8$ grid was shifted in all three directions relative to the MRI images accordingly to ensure optimal voxel placements. The MR signals of those voxels were then extracted, apodized (5 Hz Gaussian), and modeled in the time domain. A generalized linear regression model (PROC GENMOD; SAS) with PE, PC, GPE and GPC as the dependent variables, and with subject group, gender and side (right vs. left side) as the main effects, was used. A second model with the additional group-by-age interaction was included to address age effects.

Results: In the striatum of ADHD children compared with HC, PE levels were significantly lower and GPC levels tended to be higher ($p = 0.040$ and $p = 0.069$, respectively). Regarding prefrontal cortices, PE levels were significantly higher and GPC tended to be higher in the dACC of ADHD children compared with HC ($p = 0.0056$ and $p = 0.068$, respectively). In terms of age effects, there was a significant

group-by-age interaction for PE in the dACC ($p = 0.019$), PC in the iFG ($p = 0.026$) and PE in the sPL ($p = 0.0056$), all with converging values in the younger subjects and diverging values with increasing age.

Discussion: In healthy development, PE levels decrease with age reflecting a possible reduction in the demand MPL synthesis of neuronal and synaptic processes. In contrast, the other MPL precursor PC behaves differently by showing increasing levels followed by decreasing levels with age (i.e., similar to the inverted "U") at least in prefrontal cortices, which appear to reflect growth spurts in these later developing brain areas. Therefore, the lower PE in subcortical areas may reflect reduced density of dendrites and synaptic connections, which is consistent with an underdeveloped subcortical area in young ADHD children. In the dACC, the non-significant difference of PE levels in the younger subjects followed by a lack of progressive decreasing PE levels in ADHD with age compared with HC suggests a deviation in the developmental trajectory. Lastly, the increasing PC levels in the iFG of HC children are consistent with that of prefrontal areas experiencing a developmental growth spurt, which is under-achieved in the ADHD children. Ultimately, longitudinal measurements will be required to definitively address whether developmental trajectories in the brain biochemistry are altered in pediatric ADHD.

¹Psychiatry Res. 2006; 148:217-221. ²Arch. Gen. Psychiatry 2008; 65 (12): 1419-1428.

Disclosure: J. Stanley: None. D. Khatib: None. R. Dick: None. O. McGarragle: None. F. MacMaster: None. A. Robin: None. D. Rosenberg: None.

164. Antidepressant- And Anxiolytic-like Effects Following Administration Of A Brain-selective Prodrug Of 17β -estradiol (DHED) In The Mouse: Implications For Hormone-related Depression And Anxiety In Women

Michal Arad*, Laszlo Prokai, Katalin Tatrai-Prokai, Istvan Merenthaler, Todd D. Gould

University of Maryland, Baltimore, MD

Background: Women are twice as likely to experience major depression compared to men. Reductions in hormones along the cycle, following pregnancy, and around the perimenopausal period, have been associated with cognitive impairments, psychotic symptoms, and an increased risk for mood and anxiety disorders. Hormonal replacement therapy with estrogens has been suggested as a treatment for such hormone-related neuropsychiatric diseases. Although the antidepressant capacities of various estrogens have been repeatedly shown in animals and humans, women cannot benefit from chronic estrogen treatments due to potentially detrimental peripheral side effects. Previously we have shown, that a novel bioprecursor/prodrug of 17β -estradiol (E2) (called DHED by its chemical acronym) is selectively activated into E2 only in the mammalian brain; produg activation does not occur in the blood and peripheral tissues including uterus, breast, or the pituitary gland. Therefore, E2's central action after systemic DHED treatment is restricted only to the brain without exposing the periphery to the hormone. The current studies were designed to test the utility of our brain-selective prodrug approach to modify depression- and anxiety-like behaviors in the mouse.

Methods: Female C57BL/6J mice were ovariectomized at seven weeks of age. Two to three weeks later, mice were tested in several procedures measuring depressive- and anxiety-like behaviors: Forced Swimming Test (FST), Tail Suspension Test (TST), Learned Helplessness (LH), Open Field (OF), and Elevated Plus Maze (EPM). Mice were administered subcutaneously with 0, 1, 3 or 10 $\mu\text{g}/\text{kg}/5\text{ ml}$ of DHED or 25 $\mu\text{g}/\text{kg}/5\text{ ml}$ of E2 in acute (a single injection 2 hrs prior to testing), sub-chronic (daily injection for 5 days), and chronic (daily injection for 10 days prior to the first behavioral testing) paradigms.

Results: Acute administration, 2 h prior to testing, of DHED as well as E2 resulted in reduced immobility time in the FST compared to vehicle-treated ovariectomized mice. Reduced immobility in the FST

was also observed following a sub-chronic administration of five consecutive days (last injection was administered 2 h prior to testing). Following the sub-chronic administration, a clear dose-response curve was observed indicating statistical superiority for the 3 µg/kg of DHED over the other doses of DHED as well as E2. Chronic administration of DHED, at 3 µg/kg, had no effect on locomotor activity in the OF. However, DHED treatment increased the time animals spent in the center of the field as well as in the open arms of the EPM. Supporting the results observed following acute and sub-chronic administrations, chronic administration of DHED resulted in reduced immobility time in the TST and reduced despair-like behavior in the LH procedure. As expected, in all procedures the effects of DHED treatment closely resembled the effects of the parent E2. Moreover, as predicted by the CNS-specific prodrug activation to the parent E2, chronic administration of DHED had no effect on uterus weight, while E2-treated animals showed significantly increased uterus weight compared to vehicle or prodrug treated ovariectomized mice.

Discussion: The current findings indicate that in ovariectomized female mice DHED treatment exerts robust antidepressant- and anxiolytic-like activities. Given that a chronic treatment with estrogens might promote a risk for breast and uterine cancer in some women, the use of the unique CNS-specific DHED prodrug that is devoid of E2-related side effects in the periphery may provide a novel and promising treatment for women who suffer from depression and anxiety. These results bear important implications for the clinical progression and treatment of mood disorders and anxiety in women, especially when symptoms can be related to changes in hormonal level.

Disclosure: M. Arad: None. L. Prokai: None. K. Tatrai-Prokaić: None. I. Merchenthaler: None. T. Gould: None.

165. Orexins Regulate Adaptation To Repeated Stress

Seema Bhatnagar*, Willem Heydendaal

Children's Hospital of Philadelphia, Philadelphia, PA

Background: The Orexins (also called hypocretins), OrexinA and OrexinB, are synthesized exclusively in cells of the lateral and posterior hypothalamus (deLecea et al., 1998; Sakurai et al., 1998). Orexins bind to two G-protein coupled receptors, Orexin1 receptors (Orexin1R) and Orexin2 receptors (Orexin2R) to promote arousal, a heightened state of alertness or responsiveness to sensory inputs, and to promote wakefulness. An obvious extension of the role of Orexins in initiating arousal is mediating defensive responses to stressful and aversive stimuli. These defensive responses include increased anxiety states as well as increased responsiveness of the hypothalamic-pituitary-adrenal (HPA) axis. Indeed, evidence indicates that intraventricular or systemic Orexin administration increases increased anxiety state and activity of the HPA axis at all levels. Conversely, Orexin knock out mice exhibit reductions in social stress-induced increases in blood pressure, heart rate and locomotor activity. However, little is known about specific sites at which Orexin acts to regulate defensive responses. One brain region important for defensive responding is the posterior paraventricular nucleus of the thalamus (pPVT). It is an interface between sensory inputs and limbic and cortical structures of the extended amygdala (Turner and Herkenham, 1991) and our previous studies have indicated that the pPVT is important for neuroendocrine and behavioral adaptations to repeated stress. The PVT receives dense Orexin innervation, expresses moderate to high densities of both receptors and Orexins depolarize neurons of the PVT. However, whether Orexin inputs to the pPVT are important for adaptation to stress is not known. We hypothesized that Orexins acting in the pPVT mediate adaptation of defensive responses to stress.

Methods: In Experiment 1, the effects of acute microinjections of OrexinA (0.1 ng/0.2 µl) or the Orexin receptor antagonist (SB334867 at 0.1 µg/0.2 µl) into the pPVT of adult male rats were assessed on behavior in the elevated plus maze or open field or on HPA responses to acute restraint. Experiments 2 and 3 examined the role of Orexins in an important adaptation to repeated stress: the facilitated response to

novel stress (restraint) that occurs in repeatedly stressed animals (swim for 15 min/d for 4 days). In Experiment 4, the distribution of Orexin1R and Orexin2R in the cytosolic and membrane fractions of pPVT homogenates was assessed by Western blots. Finally, to assess an important indicator of cellular plasticity, phosphorylated ERK proteins in pPVT homogenates were assessed.

Results: OrexinA increased anxiety state in the elevated plus maze but had no effect in the open field or on HPA responses to acute restraint. These results suggest that Orexins are released into the pPVT upon exposure to an aversive environment to increase the anxiety state but don't decrease exploratory behavior in a novel environment or affect acute stress-induced HPA activity. Blockade of Orexin receptors by SB334867 administration into the pPVT prior to novel restraint stress did not alter the facilitated ACTH response to restraint or the increase in numbers of c-Fos expressing cells in the paraventricular hypothalamus exhibited by repeatedly swim stressed rats compared to controls. However, SB334867 administered into the pPVT prior to daily swim on four consecutive days blocked the restraint-induced facilitation of ACTH and of c-Fos expression in the paraventricular hypothalamus and prevented the repeated swim-induced increase in CRH mRNA in the paraventricular hypothalamus. SB334867 had no effect in non-stressed rats. Acute restraint did not change the distribution of either Orexin receptor compared to nonstressed rats. However, when repeatedly swim stressed rats were exposed to restraint, the Orexin1R was increased in the cytosolic compared to membrane compartment whereas the distribution of Orexin2R was not altered. Restraint alone did not increase either isoform of pERK but both pERK1 and pERK2 were markedly increased by restraint in repeatedly swim stressed rats.

Discussion: In sum, the results suggest that 1) Orexins released by daily stress into the pPVT are important for adaptation to repeated stress 2) pPVT Orexin1R internalization mediates the effects of Orexins on stress reactivity and 3) that these changes are mediated via pERK signaling. Therefore, Orexin actions in the pPVT represent an important neural substrate by which organisms adapt to stress and these actions have relevance for understanding psychopathologies associated with the inability to adapt to stress.

Disclosure: S. Bhatnagar: None. W. Heydendaal: None.

166. DPN, A Selective Estrogen Receptor-β Agonist, Activates Distinct Components Of Serotonin Circuitry To Decrease Anxiety

Lyngine Calizo*, Evan Goldart, Sheryl Beck

The Children's Hospital of Philadelphia, Philadelphia, PA

Background: Anxiety disorders diminish an individual's capacity to cope with daily life, with women having double the prevalence of men. This sex difference appears during adolescence and continues through the reproductive years, suggesting a role for gonadal hormones, such as estrogen. Estrogen can increase or decrease anxiety, and may mediate its effects through the serotonin (5-HT) system. The majority of 5-HT neurons are found in the raphe nucleus, which projects throughout the forebrain. Estrogen receptor-α (ERα) and ERβ, the two main ER subtypes, are found in 5-HT and non-5-HT neurons throughout the raphe. Studies suggest that ERα activation increases anxiety, whereas ERβ activation decreases anxiety. Estrogen impacts several components of 5-HT neurotransmission. However because estrogen binds both receptor subtypes, it remains to be determined whether any particular estrogen-induced change on 5-HT circuitry can be attributed to activation of either ERα or ERβ. These distinctions have important implications in understanding how selective activation of ERα or ERβ impacts specific components of 5-HT circuitry within the raphe to regulate anxiety. The goal of these studies is to systematically examine how estrogen selectively regulates the cellular properties and synaptic input to 5-HT raphe neurons and the specific role of a particular estrogen receptor subtype (ERβ) on this circuit in relation to behavior. We hypothesized that estrogen and DPN (ERβ agonist) have distinct effects on intrinsic cellular characteristics and

signaling onto 5-HT neurons which may have implications for their role in anxiety.

Methods: Transgenic mice with yellow fluorescent protein-labeled 5-HT neurons (YFP-5-HT) were used in all studies and facilitated targeting of 5-HT cells in electrophysiology studies. Adult YFP-5-HT mice were ovariectomized, and one week later implanted with silastic capsules containing vehicle (veh) or estradiol benzoate (EB, 50 µg in 30 µl sesame oil), a treatment regimen that increases anxiety in mice (Morgan and Pfaff, 2001). Two weeks after implantation, 200 micron slices of raphe were prepared. YFP-5-HT cells were approached individually with the electrode and whole-cell recordings obtained. Cell characteristics were recorded using current clamp techniques. The cell was clamped at -60 mV and inhibitory postsynaptic currents (IPSC) recorded under voltage clamp and isolated with the addition of DNQX (20 µM). To confirm the IPSCs were GABAergic, bicuculline (20 µM) was added at the end of the experiment. A separate group of animals was subjected to the same hormonal treatment and underwent open field testing. Another set of YFP-5-HT mice were ovariectomized and implanted with capsules containing either veh or DPN (25 µg in 30 µl sesame oil). One week after implantation mice were tested on the elevated plus maze and then 48 hours later on the open field test. Approximately 3 weeks after testing, slices were prepared for electrophysiological experiments (see above).

Results: Estradiol (EB) altered the intrinsic cellular characteristics of 5-HT neurons within the ventromedial dorsal raphe to increase their excitability. 5-HT neurons from EB-treated mice exhibited a more negative action potential threshold and increased action potential amplitude than 5-HT neurons from oil treated mice. Consistent with this, EB increased the number of action potentials generated at a given current intensity in 5-HT vmDR neurons. EB also increased sIPSC frequency thus enhancing inhibitory GABAergic, but not glutamatergic, input to vmDR 5-HT neurons. EB-treated animals showed increased anxiety in the open field. DPN did not alter cellular characteristics and failed to enhance the excitability of vmDR 5-HT neurons. The number of action potentials generated in response to a given current intensity did not differ between veh- and DPN-treated animals. However, as seen by an increase in sIPSC frequency, DPN enhanced inhibitory GABAergic input onto 5-HT vmDR neurons. DPN-treated animals showed decreased anxiety.

Discussion: Activation of both ER α and ER β (estradiol) increased intrinsic excitability and inhibitory GABAergic input onto 5-HT neurons. Use of a selective ER β agonist, DPN, revealed that ER β activation alone was responsible for the enhanced GABA input, which in turn may decrease anxiety. Thus activating ER α /ER β together or ER α alone may enhance the excitability of 5-HT cells, which may result in increased anxiety. Together these results from EB or DPN suggest that selective activation of particular ER subtypes impacts specific components of 5-HT circuitry within the raphe to regulate anxiety.

Disclosure: L. Calizo: None. E. Goldart: None. S. Beck: None.

167. Roles of Estrogen Receptors Alpha and Beta in Sexually Dimorphic Neuroprotection Against Glutamate Toxicity

Daniel Dorsa*, Damani Bryant

Oregon Health and Science University, Portland, OR

Background: Although most agree that estrogens are neuroprotective via a variety of mechanisms, less is known about whether this effect is equivalent between the sexes. Furthermore, it is not yet clear exactly which receptors are critical for estradiol neuroprotection, and whether they are the same in the male and female brain. In the present study, we compared the ability of short term estrogen exposure to protect sex specific rat primary cortical neurons from a cytotoxic insult. As estrogen was found to effectively protect female, but not male-derived cells, we also examined the potential roles of estrogen receptors alpha (ER α) and beta (ER β), as well as other estrogen receptors in this difference.

Methods: To address this issue, we isolated primary cortical neurons from rat pups sorted by sex and assessed the ability of estradiol to

protect the neurons from death induced by glutamate using the Live/Dead Assay. Western Blots were used to document levels of expression of ER α and ER β . Some cultures were pretreated with selective agonists or antagonists of both "nuclear" ERs, as well as other putative membrane ER agonists such as STX and G1.

Results: A short term five minute pretreatment with 10 to 50 nM 17 β -estradiol, previously used to identify membrane-based signalling, protected female but not male neurons from glutamate toxicity 24 hours later. Experiments using an ER α selective agonist or antagonist indicate that this receptor is a primary contributor to neuroprotection in female cortical neurons. The ER β selective agonist conveys a small degree of neuroprotection to both male and female cortical neurons. Interestingly, we found that 17 α estradiol and the novel membrane estrogen receptor (mER) agonist STX, but not bovine serum albumin conjugated estradiol or the GPR30 agonist G1 were neuroprotective in both male and female neurons.

Discussion: We have documented a clear sex difference in estrogen's ability to protect cortical neurons from cytotoxic insult. This "dimorphism" apparently is present prior to that induced in the brain by the early post natal exposure of the brain to testosterone/estradiol. Expression of both "nuclear" receptors was evident in cultures from both sexes, but it appears that ER α is the predominant mediator of estrogen's effects. Activation of ER β was also protective when ER alpha was blocked. As we and others have documented that both ER α and ER β can serve as membrane ERs, our data do not rule out this mode of action. Interestingly, however, the mER agonist STX protected neurons from both sexes equally, suggesting that receptor activation profiles may differ between estradiol and STX. Taken together these data document an inherent difference in protective actions of estrogen based on genetic sex, and highlight a role for ER α in this sexually dimorphic effect.

Disclosure: D. Dorsa: None. D. Bryant: None.

168. Bcl-2 Associated Athanogene (BAG-1)/FKBP51 Complex Regulates GR Trafficking to the Mitochondria: Potential Role in Regulating Affective Resilience

Jing Du*, Yun Wang, Ilia Karatsoreos, Yanling Wei, Rayah Blumenthal, Rayah Blumenthal, John Reed, Bruce McEwen, Manji Husseini

NIMH, Bethesda, MD

Background: Glucocorticoids play an important biphasic role in modulating mitochondrial functions and synaptic plasticity. This mechanism may be involved in the pathophysiology of stress and stress-related mental illnesses. BAG-1, a downstream target of lithium and valproate, interacts with PTSD- and depression-associated gene FKBP51, glucocorticoid receptors (GRs), Bcl-2, heat shock protein 70, and inhibits GR receptor nuclear translocation in response to corticosterone (CORT).

Methods: We studied BAG-1 and FKBP51 levels in the mitochondria and BAG-1/FKBP51 interaction after CORT treatment in cortical neurons. We also investigated whether over-expression of Bag-1 could protect CORT treatment induced reduction of GR and Bcl-2 levels in the mitochondria and the anhedonic-like behaviors *in vivo*.

Results: We found that BAG-1 and FKBP51 distribution in the mitochondria was significantly increased in response to CORT treatment in cultured cortical neurons; interestingly, similar effects were seen with GR and Bcl-2 mitochondrial levels after CORT. Furthermore, FKBP51 formed a complex with BAG-1, and the formation of FKBP51 and BAG-1 complex increased after CORT treatment. However, total BAG-1 protein expression was decreased in neuronal homogenates, suggesting a complex regulation by CORT. Notably, BAG-1 overexpression in BAG-1 transgenic mice not only blocked the reduction of GR/Bcl-2 in the mitochondria, but also the formation of anhedonic-like behavior after CORT treatment. Wild type FVB mice showed a reduction in saccharine consumption after CORT treatment; however, BAG-1 transgenic mice did not, suggesting a resilience to high dose CORT.

Discussion: These data suggest that the regulation of the trafficking of key plasticity molecules to the mitochondria may play an important role in the mechanisms by which chronic stress and glucocorticoids regulate cellular plasticity and resilience, and ultimately sensitivity/resilience to the development of stress-induced psychiatric disorders.

Disclosure: J. Du: None. Y. Wang: None. I. Karatsoreos: None. Y. Wei: None. R. Blumenthal: None. R. Blumenthal: None. J. Reed: None. B. McEwen: None. M. Husseini: None.

169. Disruption of Spatial Working Memory Component Processes by Electroconvulsive but not Magnetic Seizure Therapy

Shawn McClintock*, Nick DeWind, Herbert Terrace, Mustafa Husain, Timothy Spellman, Stefan Rowny, Sarah H. Lisanby

UT Southwestern Medical Center, Dallas, TX

Background: Experiments on spatial working memory use a variety of paradigms that require subjects to maintain a representation of particular locations throughout each trial. Free-range spatial measures are advantageous as they provide unique information regarding the subject's strategy in completing the task. For example, one such strategy to accurately complete the spatial working measure is stereotypy. Stereotypy involves the sequential selection of a spatial pattern in a repetitive manner, once a correct sequence has been identified. The use of such a strategy on self-ordered spatial working memory tasks has been reported in human studies, and one prior nonhuman primate study. In prior experiments, we developed a battery of touch-screen, computerized, neurocognitive measures, the Columbia University Primate Cognitive Battery (CUPCB), that assess short-term, long-term, and working memory. The CUPCB was used in an investigation that examined the effects of electroconvulsive therapy (ECT), magnetic seizure therapy (MST), and sham intervention (anesthesia only), and found that ECT decreased accuracy on the spatial working memory task, but MST did not differ from sham. Here we examine the effects of ECT and MST on stereotypy in order to elucidate if it was differentially affected by ECT, with the hypothesis that it would be disrupted by ECT, but not MST.

Methods: In a within-subject study design, we assessed the effects of ECT, MST, and sham on reaction time and stereotypy in a preclinical model. The subjects were three pathogen-free male macaca mulatta with a mean age of 83 ± 26 months. Subjects completed the free-range spatial working memory measure of the CUPCB. Stereotypy was assessed by computed correlations of actual and predicted touch patterns of the spatial stimuli. To accomplish this, we examined each subject's performance during the baseline period. Each baseline trial consisted of two to six responses, and we computed the mean ordinal position of the responses for each of the 16 ports. We rank ordered each port for each subject based on the mean ordinal position of responses made at that port within the baseline trials. We then calculated Kendall tau correlations between the rank order assigned to a particular port and the actual ordinal position it was touched within a trial for each experimental condition, and separately for correct and incorrect presses. Confidence intervals were bootstrapped by resampling the data from each condition with replacement to create one thousand new data sets for each condition. Kendall tau correlation coefficients were recalculated for each new dataset to establish 95% confidence intervals around the correlation coefficient.

Results: For correct trials, analysis of the reaction time showed a main effect of condition ($F(1,3) = 20.6$, $p < 0.0001$), with reaction times being longer in the ECT relative to baseline (estimated difference = 0.72, 95% confidence interval [CI]: 0.31, 1.13), sham (estimated difference = 0.92, 95% CI: 0.60, 1.25), or MST (estimated difference = 0.78, 95% CI: 0.44, 1.11) conditions. There were no differences in reaction time between baseline, sham, or MST. For incorrect trials, although a similar pattern was found with longer reaction times in the ECT condition, the effect was not significant ($F(1,3) = 2.62$, $p = 0.05$). Across all trials, associations between predicted and actual touch of spatial stimuli were lower in the ECT condition ($r = .20$, 95% CI: .19, .22) compared to baseline

($r = .31$, 95% CI: .29, .33), sham ($r = .30$, 95% CI: .28, .31), and MST ($r = .31$, 95% CI: .29, .32) conditions, suggesting that it disrupted stereotypy. This similar pattern was observed when separately analyzing correct and incorrect trials.

Discussion: To our knowledge, this is the first study to examine the effects of ECT on spatial working memory component processes. Our findings suggested that ECT, but not MST, negatively impacted reaction time and stereotypy in a preclinical model. We also found that stereotypy is relatively preserved following MST, possibly due to its relatively more focal effects and sparing of the hippocampus in comparison with ECT. The results of this study have significant implications by elucidating how ECT may impact spatial working memory and by providing a useful computerized measure of self-ordered spatial working memory. This line of work requires replication in a clinical model as it may help improve our understanding of cognitive component processes underlying neurocognitive functions.

Disclosure: S. McClintock: Part 1; NIH. Part 2; NCRR. Part 3; NASAD. N. DeWind: None. H. Terrace: None. M. Husain: Part 1; NIH, NIDA, NINDS, NIA. Part 2; NARSAD, Stanley Foundation. Part 3; Cyberonics, Neuronetics (past). Part 4; St. Jude Medical (ANS). Part 5; Magstim, Inc. (equipment only), Brainsway. T. Spellman: None. S. Rowny: None. S. Lisanby: Part 1; Northstar DSMB (past), Magstim (equipment), MagVenture (equipment). Part 2; ANS/St. Jude Medical, Neuronetics, Cyberonics. Part 3; Brainsway, Astra Zenica (support of CME Event).

170. Cellular Activity Measured with C-Fos during Deep Brain Stimulation to the Lateral Habenula

Martine Mirrione*, Bo Li, Fritz Henn

Bookhaven National Lab., Upton, NY, Brookhaven National Laboratory, Upton, NY

Background: Chronic high frequency Deep Brain Stimulation (DBS) may offer a novel therapeutic strategy for treatment-resistant depression. However, the biological mechanisms by which DBS exerts its therapeutic effects are not fully understood. Several targets have been explored for treating depression including the subgenual cingulate cortex (area 25), nucleus accumbens/ventral striatum, inferior thalamic peduncle, and rostral cingulate cortex (area 24). Recent circuit data from our group has suggested that the lateral habenula (LHb) is an additional promising DBS target, as it controls midbrain monoamines. We have successfully applied LHb-DBS in two animal models of depression, reducing learned helplessness (LH) behavior and preventing increased immobility in the forced swim test. Furthermore, full remission was achieved in the first patient receiving bilateral LHb-DBS. Here we extend those studies by measuring the effects of DBS at the cellular level using c-fos immunohistochemistry.

Methods: In this study, 38 wild type, male, Sprague-Dawley rats were exposed to the learned helplessness paradigm (40 minutes of inescapable foot-shock, followed by an escape test 24 hours later where ability to shut off the shock with a lever press was assessed over 15 trials). The tests resulted in 17 animals meeting the criteria for LH (0–6 lever presses) which were selected for surgical implantation. Bipolar concentric electrodes with a 0.8 mm tip, were implanted unilaterally to the LHb (-3.7 mm posterior, +0.8 mm lateral, -5.0 mm ventral, coordinates from bregma). Animals recovered from surgery for 7 days and were split evenly between 3 groups, sham, DBS at 150 µA, or at 300 µA (130 Hz, 60 µs pulse width). On the day of the experiment, rats were connected to a stimulator in their home cage. Following 1 hr of DBS (or sham-connection), animals were sacrificed by perfusion with 4% paraformaldehyde, the brains removed, post fixed for 1 hr, and dehydrated in 30% sucrose. Serial sections were obtained with a cryostat (40 µm) between the prefrontal cortex and locus coeruleus. Immunohistochemistry was performed using established protocols with antibodies against c-fos (Santa Cruz) and the neuronal marker NeuN (Millipore). Alexa Fluor secondary antibodies (Invitrogen) were

used for immunofluorescent detection. Digital images were acquired at identical exposure times, and quantified with Image J.

Results: A clear pattern of c-fos expression emerged in the area surrounding the implanted electrodes. Few c-fos positive cells were present in the LHb of sham animals, whereas a 55% increase in immunoreactivity was quantified in the LHb of the 150 µA group, and a 69% increase observed in the 300 µA group. By comparison, the contralateral LHb of individual animals showed less c-fos expression compared to the ipsilateral side in both DBS treated groups. There was a significant increase in c-fos expression in the dorsal raphe nucleus (DRN) of stimulated animals (One-way ANOVA $p = 0.0003$, Tukey post-hoc) however, the difference between 150 and 300 was not significant. Ongoing studies are aimed at evaluating additional afferent and efferent projection sites including the ventral tegmental area, and locus coeruleus as well as further identifying the cell types responding to DBS.

Discussion: Our study design was meant to mimic the experimental conditions previously used to ameliorate LH behavior thus beginning to explore the mechanistic effects of LHb-DBS. We observed a dose response pattern of c-fos activity in the area surrounding the implanted electrodes where higher currents affected more cells however further studies are needed to identify these cell types and the nature of their activity changes. Recent data from the literature exploring c-fos and electrophysiological activity during DBS, showed neurons are initially depolarized and had a transient increase in spontaneous activity, but this was followed by a sustained depolarization block. Our data are consistent with the hypothesis that LHb-DBS may initially induce neuronal activity, though further studies will be necessary to dissect this effect. Several studies have shown that LHb-DRN excitability is linked to depression in humans and learned helplessness behavior. Therefore our data showing DBS to the LHb influences DRN cells may be an important component underlying circuitry changes reducing depressive-like symptoms. Overall, these studies will facilitate our understanding of LHb-DBS as we begin to uncover its effects at the level of cells and circuits.

Disclosure: M. Mirrione: None. B. Li: None. F. Henn: None.

171. The Efficacy of Three Electrode Placements in Older vs Younger Patients with Severe Depression Treated with Electroconvulsive Therapy

Georgios Petrides*, Charles Kellner, Rebecca Knapp, Mustafa Husain, Keith Rasmussen, Shirlene Sampson, Samuel Bailine, Raphael Braga, Kristen Tobias, Munro Cullum, Shawn McClintock, Martina Mueller, Max Fink

The Zucker Hillside Hospital, NS-LIJ Health System, Glen Oaks, NY

Background: Electroconvulsive therapy (ECT) is widely considered to be the most effective treatment for severe depression. Technical refinements aim to improve efficacy and minimize cognitive effects. Electrode placement is an important factor that may affect outcomes. Traditionally ECT has been performed with one of 3 electrode placements: bilateral (bitemporal) (BT), right unilateral (RUL) and bifrontal (BF). The choice of electrode placement in clinical practice is largely determined by clinical experience and clinician preference. We recently reported on a multicenter, NIMH-funded, randomized trial comparing these three electrode placements. Our data showed that each electrode placement is a very effective antidepressant treatment with similar cognitive effects. More specifically, remission rates were 64% for BT, 61% for BF and 55% for RUL. Bilateral placement resulted in more rapid symptom reduction and therefore was recommended for urgent clinical situations. There are no data supporting the preferential use of a particular placement in older vs younger populations. In this report we examine the efficacy of the 3 electrode placements on depressed patients older and younger than 65 years of age.

Methods: In a multicenter, NIMH-supported clinical trial, 230 patients were randomized to receive RUL, BF, or BT ECT. Patients were

diagnosed with a major depressive episode (unipolar or bipolar) by SCID-IV and had an entry 24-item Hamilton Rating Scale for Depression ($HAMD_{24} \geq 21$ (mean = 35). A neuropsychological test battery was administered at five specified time points. Patients and raters were blinded to electrode placement. Stimulus dose titration at the first ECT was followed by dosing at 1.5X Seizure Threshold (ST) for BF and BT, and 6X ST for RUL. Remission was defined as 2 consecutive $HAMD_{24} \leq 10$. Dropouts and those who did not complete the protocol were considered non-remitters. We analyzed the data looking at differences between patients 65 years of age and older, and younger patients.

Results: Bitemporal ECT was similarly effective in both age groups. Remission rates were 72% for patients older than 65 and 61% for younger patients. With RUL there was a statistically significant difference in the proportion of remitters in the groups, with 74% of the older group responding and 48% of the younger group ($p = 0.05$). With BF there was a trend suggestive of better outcome in the younger group (65% vs 44%, $p = 0.1$). In a direct comparison between BF and RUL, there was a trend suggesting superiority of BF in the younger group (65% vs 48%, $p = 0.06$) and RUL in the older group (44% vs 75%, $p = 0.07$).

Discussion: Our data suggest that bifrontal ECT at 1.5 times ST is more effective in patients younger than 65 yo compared to older patients. Right unilateral ECT at 6 times ST is more effective in patients 65 and older, compared to younger patients.

Disclosure: G. Petrides: Part 4; Corcept, Novartis, Proteus, Merck. C. Kellner: None. R. Knapp: None. M. Husain: None. K. Rasmussen: None. S. Sampson: None. S. Bailine: None. R. Braga: None. K. Tobias: None. M. Cullum: None. S. McClintock: None. M. Mueller: None. M. Fink: None.

172. Extracellular Levels of Leucine Enkephalin in the Rat

Dorsolateral Striatum: Mass Spectrometric Quantification

Brian Reed*, Yong Zhang, David Fenyo, Brian T. Chait, Mary Jeanne Kreek

The Rockefeller University, New York NY

Background: Methionine and leucine enkephalin are two principal endogenous opioid peptides derived from preproenkephalin, which is expressed in striatopallidal neurons. Previous attempts to quantify enkephalin levels in the brain have relied on antibodies, coupling microdialysis to radioimmunoassay; the low levels of peptide as well as the lack of absolute specificity of the antibodies make such studies technically difficult at best, generally requiring the use of chromatographic separation for verification of signal. Mass spectrometric detection has long been recognized as having the potential for vastly improving the specificity of detection, but the promise of endogenous neuropeptide detection using mass spectrometry has until only recently proven elusive. The vast complexity of biological fluids such as plasma, cerebrospinal fluid, and microdialysis fluid, with the sheer number and overall concentration range of different molecular entities, has prevented simple single stage or tandem mass spectrometry with data dependent fragmentation from being generally effective in the detection of peptide hormones with concentrations in the sub-nanomolar range. We recently employed targeted multistage (ms^3) mass spectrometry for the semiquantitative detection of vasopressin and oxytocin in plasma, the first demonstration of this technique for the detection of endogenous peptidic hormones in plasma. Other laboratories have recently employed similar methodology to detect neuropeptides in the microdialysis fluid from the rat striatum. The current work extends these methods, utilizing stable isotope dilution mass spectrometry for the unambiguous quantification of leucine enkephalin in microdialysis fluid.

Methods: Fischer rats were stereotactically implanted with microdialysis probe guide cannulae in the striatum, and allowed to recover for 72–96 hours; the probe (CMA/12, 2 mm) was implanted the night before the experiment. Microdialysis was carried out with artificial

cerebrospinal fluid perfused at a rate of 2 μ l/min, with samples collected for 20 minutes. Stable isotope standards, ^{13}C -[Gly₂]-leucine enkephalin and ^{13}C -[Gly₂]-methionine enkephalin, were added to microdialysis samples; samples were acidified by 1:1 dilution with 2% TFA and loaded manually onto 75 μ m i.d. capillary C18 columns, from which they were directly introduced to a LTQ ion trap mass spectrometer. Both Leu- and Met-enkephalin give rise to a predominant a₄ ion, 397.7 m/z, which was targeted in ms³ mode, with the resulting promising b₃ ion, 323.1 m/z used for quantification (with other transitions used for verification purposes); comparison with the integrated intensity of the corresponding transition for the stable isotope standard was used for quantification.

Results: In the recovered microdialysis fluid of Fischer rat, the baseline level of leucine enkephalin was found to be 26 (\pm 5) pM. Treatment with 15 mg/kg cocaine did not result in any significant changes in recovered leucine enkephalin levels (at time points for which we and others have previously demonstrated increases in extracellular dopamine levels). In these initial studies, quantification of methionine enkephalin was problematic due to variable oxidation of the C-terminal methionine residue both in the endogenous peptide, as well as for the standard; oxidative treatment of the microdialysate following stable-isotope standard addition may allow us to address this issue.

Discussion: Studies using antibodies performed in a rigorous fashion using chromatographic characterization of immunoreactivity have previously been performed and demonstrated the presence of extracellular enkephalin in the striatum. The development of mass spectrometric methods of quantification offers advantages in terms of specificity and methodological flexibility in comparison with radioimmunoassay. Our studies demonstrate the use of targeted multistage mass spectrometry for the quantification of endogenous leucine enkephalin in rat microdialysis fluid. The lack of alteration of leucine enkephalin following cocaine administration is consistent with the relative ineffectiveness of naltrexone in attenuating the effects of cocaine; additional studies utilizing our novel methodology, incorporating additional cocaine doses, timepoints and neuropeptides of investigation, as well as other psychostimulant drugs, will be useful in understanding the role of neuropeptides in cocaine abuse. The mass spectrometry methods used here will also allow us to unambiguously quantify other neuropeptides, and can also be applied to investigations of plasma levels of peptidic hormones.

Disclosure: B. Reed: None. Y. Zhang: None. D. Fenyo: None. B. Chait: None. M. Kreek: None.

173. Feasibility Study of Serum Biomarker Panels for the Diagnosis of Major Depressive Disorder and the Prediction of Treatment Outcomes

Perry Renshaw*, Sujung Yoon, Tae-Suk Kim, Jaeuk Hwang, Katie Smith, John Bilello, Bo Pi, In Kyoon Lyoo

University of Utah, Salt Lake City, UT

Background: The diagnosis of depression depends on patient self-report of symptoms. However, clinical diagnoses of psychiatric disorders and those made using structured interviews, as in research, have been shown to be quite dissimilar; Rettew et al. have estimated a kappa, based on a metanalysis of published studies, of just 0.14 for affective disorders¹. For many depressed patients, the initial treatment is often ineffective or the patient may experience side effects that lead to the cessation of treatment and initiation of one or more subsequent therapies. The need for developing a more reliable biological marker for diagnosing depression and predicting treatment responses has, therefore, been increased. In order to evaluate a laboratory based method for the diagnosis of depression, a biomarker panel was developed^{2,3}. Following the assessment of 96 possible markers in a study of 50 depressed and 50 comparison subjects, a final panel of 9 markers, including compounds from neurotrophic, metabolic, inflammatory, and HPA axis pathways, was selected for the diagnosis of depression. The test includes measures of: Alpha-1 antitrypsin, Apolipoprotein CIII, Brain Derived Neurotrophic Factor, Cortisol,

Epidermal Growth Factor, Myeloperoxidase, Prolactin, Resistin, and Soluble Tumor Necrosis Factor Alpha Type II (sTNFR2). This panel was validated in a subsequent study of 123 subjects (80 depressed and 43 normal). The panel discriminated patients with MDD from normal controls ($p = 5.8e-19$) and showed a clinical sensitivity of 87% and specificity of 95%. A second biomarker panel, including six markers, was designed to include compounds that were most likely to change with successful treatment. This panel was further studied in a separate cohort of depressed patients to explore the ability of the panels to predict treatment outcomes.

Methods: Depressed adult subjects were enrolled at three Medical Centers in South Korea following IRB approval of the protocol. Enrolled subjects were 18 to 65 years old, met DSM-IV criteria for Unipolar Major Depression, (single or recurrent), had a 21-item Hamilton Depression Rating Scale (HAM-D) score >18, and were capable of providing informed consent. All subjects were psychoactive drug-free at study start and had a Structural Clinical Interview for DSM-IV (SCID) at baseline. Patients were treated with escitalopram for 8 wks and provided blood samples at baseline, 2, and 8 wks post-treatment. In addition, The Hamilton Depression Rating Scale (HDRS) was assessed at baseline, and at 1, 2, 4, and 8 week visits. De-identified samples were frozen at -80°C until biomarker testing using immunoassay methods could be completed. For the diagnosis of depression, a biomarker depression score (ranging from 1-9 indicating low to high likelihood of depression) was determined.

Results: A cohort of 10 patients was enrolled in the treatment monitoring study. All patients had a biomarker depression score at baseline of 6 or higher, consistent with a moderate to high likelihood of depression, thereby correlating with study subjects' initial HAM-D ratings of >18. All experienced a significant decrease in their HAM-D score over the 8 weeks of treatment, with changes ranging from 16 to 28 HAM-D points. A second assessment was applied to determine if the week 2 panel results could predict treatment outcome at week 8. The biomarker monitoring panel (Prolactin, Brain Derived Neurotrophic Factor, Resistin, Tumor Necrosis Factor, Alpha Type II (sTNFR2), Alpha-1 antitrypsin) results at baseline and week 2 were evaluated by regression analysis with the change in HAM-D score from baseline to week 8. This analysis yielded a correlation coefficient of 0.88, suggesting that the monitoring biomarker panel values at week 2 may have the potential to predict monotherapy treatment outcome at week 8.

Discussion: This study in a small cohort suggests the utility of multi-analyte biomarker panels for the diagnosis of depression as well as the prediction of patient response to antidepressant therapy. However, these findings are limited by the small sample size and larger studies in well-defined depressed patient populations will be needed to validate these early observations. References: 1. Rettew D, et al. Int J Methods Psychiatr, 2009, 18(3): 169-184. 2. Renshaw P, et al. American Psychiatric Association Meeting May, 2009, poster NR7-014. 3. Bilello J, et al. U.S. Psychiatric and Mental Health Congress. November, 2009, poster #106.

Disclosure: P. Renshaw: Part 1; Novartis, Roche, Kyowa Hakko, Ridge Diagnostics. Part 4; Roche, GlaxoSmithKline. S. Yoon: None. T. Kim: None. J. Hwang: None. K. Smith: Part 1; Ridge Diagnostics. J. Bilello: Part 5; Ridge Diagnostics. B. Pi: Ridge Diagnostics. I. Lyoo: Part 4; GlaxoSmithKline, Eli Lilly.

174. In-Silico Homology Prediction, Post-translational Modifications and Effects on Tertiary Structure of Human Proteins Affected by Mood Stabilizer Treatment Based on Rat Synaptoneurosomal Preparations from the Pre-frontal Cortex: An Inexpensive Approach for Drug Target Discovery and Validation

Natalia Lorena Gorron, Maria Corena-McLeod, Elliott Richelson*

Mayo Clinic, Jacksonville, FL

Background: Sequence homology between rat and human proteins allows determination of protein identification in one species based on a comparison of primary sequence from the other. Many of these proteins require post-translational modification by specific enzymes to

become functional. These modification sites are under dual selective constraint. They must assume the conformation necessary to become modified by the activating enzyme as well as the dynamic conformations required for their proper function. There are significant functional modifications associated with particular homology domains that have evolved with such constraints between species. Those conserved domains are the basis for structure determination, drug discovery, as well as genomic and proteomic factors influencing disease.

Methods: We isolated synaptoneuroosomal preparations from rat prefrontal cortex (PFC) after chronic lithium, valproate, and paliperidone treatment. We separated proteins using 2D-DIGE and identified those that were differentially expressed and phosphorylated after drug treatment. Selected proteins were identified by nano-LC-ESI MS/MS. Sequence homology to human proteins was determined using BLASTP. Phosphorylation and homolog comparison was performed using Phosphosite Plus®. Location of predicted phosphorylated spots and degree of phosphorylation was determined using changes in isoelectric point (pI) and image analysis. Prediction of relevance to gene expression in humans was obtained through Gene Expression Omnibus (GEO) profiles based on clinical data of controls versus bipolar or schizophrenic patients. Protein interactions and pathways were identified using Biosystems and KEGG. Tertiary structural differences were identified using Cn3D. Unidentified protein location was mapped in 2D-DIGE gel images using image comparisons generated from different sets of experiments. Drug Bank was used to determine drug properties and structural homologs of drug candidates. Virtual docking was performed when appropriate.

Results: Our results indicate that human proteins homologous to those identified in rat synaptoneuroosomal preparations from the PFC share the same function and sequence with the exception of two proteins (VATA and DESP homolog). We identified phosphorylation sites in each peptide group and predicted the degree of phosphorylation based on changes in isoelectric point (pI) and their location on 2D-DIGE gels. We were able to predict changes in expression of the corresponding human genes using clinical data obtained from bipolar or schizophrenic patient studies using GEO. 3D structure comparison was performed using cn3D and possible drug candidates were determined using Drug Bank and Dock blaster along with the possible signaling or metabolic pathway involved.

Discussion: We were able to use public free-access databases to identify human homologs of proteins identified in our rat PFC synaptoneuroosomal preparations after mood stabilizer treatment. Our results indicate that the corresponding human proteins share not only sequence homology and function but also similar post-translational modifications and tertiary structure. The relevance of these comparisons relies on the involvement of these molecules in common signaling and metabolic pathways and the implications for development of novel drugs for treatment of neuropsychiatric disorders. In addition, new uses for existing drugs were revealed. Significance of gene expression in human clinical data revealed the importance of this kind of comparison using multiple platforms. This methodology is particularly useful in limited internet and computer access settings. In addition, it can be used by downloading stand-alone versions of the databases in institutions with limited internet access in developing countries. (Supported in part by Mayo Foundation for Medical Education and Research.)

Disclosure: N. Gorron: None. M. Corena-McLeod: Part 1; Ortho-McNeill Janssen. E. Richelson: Ortho-McNeil Janssen. Part 2; Astra-Zeneca. Part 3; Sarentis Therapeutics.

175. The Afterlife of Brain Mitochondria: Postmortem Studies in Mouse and Human

Keri Barksdale, Emma Perez-Costas, Johanna Gandy, Miguel Melendez-Ferro, Rosalinda Roberts*, Gautam Bijur

University of Alabama, Birmingham, Birmingham, AL

Background: Brain mitochondria have multiple critical functions, and are the major source of ATP for neurons. Many studies have reported the

occurrence of altered or decreased mitochondrial function in the aging brain, in neurodegenerative diseases such as Alzheimer's disease, in psychiatric disorders such as schizophrenia, and in neurometabolic disorders such as Leigh syndrome. Thus, it is clear that mitochondria play a fundamental role in normal brain function and in brain disease processes. To assess mitochondrial activity in human brain, investigators have previously utilized frozen postmortem brain tissues to analyze mitochondrial enzymatic activities, mitochondrial protein levels, and mitochondrial DNA. However, there are several other vital indices that could provide insight into brain mitochondrial activity that cannot be conducted in frozen tissue samples. These include measurements of the mitochondrial membrane potential, ATP production, calcium buffering capacity, and respiration, which together give an overall assessment of mitochondrial health and activity. Postmortem human brains are potentially an abundant source of mitochondrial material. However, it is generally thought that direct measurements of mitochondrial activity, such as mitochondrial transmembrane potential and ATP production are not possible in postmortem brain samples. This is because of the common belief that organelle functions cease after death. In the present study we chose to challenge this concept and study the activity of mitochondria derived from postmortem brain tissue.

Methods: Postmortem brain tissue was obtained from the Alabama Brain Collection. C57/Bl6 mice were obtained commercially. All experiments were conducted in accordance with the UAB IACUC or IRB approved protocols. To test the effect of postmortem interval (PMI) on mitochondrial viability, mice were euthanized by cervical dislocation and intact bodies were stored at 4°C for 0, 10, 18, or 24 hours after death. Mitochondria were isolated from cerebral cortex, and functional assays for membrane potential and ATP production were conducted in fresh and cryopreserved mitochondria. To study structural integrity, electron microscopy was performed on isolated mitochondrial pellets.

Results: The mitochondrial transmembrane potential and ATP production can be engaged in mitochondria isolated from human brains up to 8.5 hours postmortem. Also, a time course of postmortem intervals from 0 hours to 24 hours using mitochondria isolated from mouse cortex revealed that mitochondrial transmembrane potential can be reconstituted beyond 10 hours postmortem. Furthermore, mitochondria isolated from mouse brains up to 10 hours postmortem and human brains up to 8.5 hours postmortem maintain ATP-producing capability; however, ATP production rates decrease with longer postmortem intervals. Lastly, postmortem brain mitochondria retain their structural integrity as well as their transmembrane potential and ATP production capacities following cryopreservation.

Discussion: In this study, we report that postmortem mouse and human brains with relatively short PMIs are an abundant source of structurally intact and functional mitochondria, and mitochondria can be stored frozen for functional measurements at a later time. Our findings that transmembrane potential and ATP generating capacity can be reinitiated in brain mitochondria hours after death indicates that human postmortem brains can be an abundant source of viable mitochondria to study metabolic processes in health and disease, and it is also possible to archive these mitochondria for future studies. These methods provide both a novel finding of functional mitochondria from postmortem tissue and extremely valuable tools for the future understanding of how mitochondria may be affected in familial and sporadic brain disorders.

Disclosure: K. Barksdale: None. E. Perez-Costas: Part 4; UAB, NIMH. J. Gandy: None. M. Melendez-Ferro: UAB, NIMH. R. Roberts: UAB, NIMH. G. Bijur: UAB, NIMH.

176. Linking Increases in Functional MRI Signal Evoked by Ketamine to Changes in Extracellular Glutamate Efflux-Modulation by Gabapentin, Lamotrigine, and LY379268, and Therapeutic Implications for Psychotic Disorders

Scott Schobel*, Nashid Chaudhury, Cheryl Corcoran, Nicole Lewandowski, Jeffrey Lieberman, Holly Moore, Scott Small

Columbia University Department of Psychiatry, New York, NY

Background: Hippocampal hypermetabolism as assessed by PET or high-resolution basal-state functional imaging characterizes

schizophrenia, is associated with clinical symptoms of psychosis, and predicts clinical progression to psychotic disorders from prodromal stages of disease with a positive predictive value of .70 and negative predictive value of .88 based upon recent preliminary studies. Though hippocampal hypermetabolism has been evoked in the acute NMDA antagonist model using *ex-vivo* audioradiographic and *in-vivo* functional MRI techniques, the presence of a hypo vs. hyperglutamatergic state within hippocampus remains undetermined. Prior empirical studies which have directly measured glutamate efflux in the NMDA antagonist model, conducted exclusively in rodent cortex, have shown evoked increases in extracellular glutamate concentration. Pinpointing the nature of this neurochemical abnormality within hippocampus has therapeutic implications for glutamate modulating strategies in schizophrenia and, potentially, for preventative strategies in prodromal stages of psychotic disorders.

Methods: To directly explore the relationship between evoked functional MRI signal and extracellular glutamate efflux within hippocampus, we exposed C57b6 WT mice to acute ketamine challenge (30 mg/kg) or saline (n = 9 per group) during high resolution functional MRI (CBV) of hippocampal subregions (100 μ M resolution), as well as during recording of extracellular glutamate efflux within hippocampal subregions via *in-vivo* voltammetry (500 msec time resolution and sub-micromolar sensitivity) and measured the evoked response of both variables. In a second set of experiments, in order to determine whether evoked functional MRI signal and extracellular glutamate abnormalities within hippocampal subregions could be manipulated by modulation of the glutamate neurotransmitter system, mice were pre-treated for five days with either gabapentin 600 mg/kg, lamotrigine 10 mg/kg, LY379268 10 mg/kg, or saline, and then challenged with ketamine during functional MRI or recording of extracellular glutamate efflux.

Results: Ketamine challenge evoked rapid increases in CBV within the CA1 ($F_{4,13} = 4.1$, $p = .02$), subiculum ($F_{4,13} = 3.8$, $p = .03$), and dentate gyrus ($F_{4,13} = 4.6$, $p = .02$) subregions, with non-significant changes found in the CA3 ($F_{4,13} = 1.4$, $p = .28$) and entorhinal cortex ($F_{4,13} = 2.4$, $p = .11$). Similarly, ketamine challenge evoked rapid increases in extracellular glutamate within the CA1 subfield (peak amplitude $1.49 \mu\text{M} \pm 1.23$, $t_8 = 2.6$, $p = .03$), subiculum ($1.36 \mu\text{M} \pm 1.23$, $t_{10} = 2.5$, $p = .03$), and dentate gyrus granular cell layer ($1.64 \mu\text{M} \pm 1.1$, $t_5 = 2.6$, $p = .05$) subregions, with non-significant changes found in the CA3 subfield ($.86 \mu\text{M} \pm 1.14$, $t_3 = 1.38$, $p = .26$) and slight decreases observed in medial entorhinal cortex ($-.71 \mu\text{M} \pm 1.24$). Ketamine evoked abnormalities in the CA1 subfield, subiculum, and dentate gyrus were successfully blocked by pre-treatment with gabapentin, lamotrigine, or LY379268.

Discussion: The present results link ketamine-evoked increases in functional MRI signal within hippocampus to increases in extracellular glutamate, and demonstrate that pre-treatment with glutamate-limiting therapies through diverse pharmacological mechanisms can block this evoked phenotype. Taken together with recent clinical findings in schizophrenia, these results suggest that increases in hippocampal metabolism as assessed by functional MRI index a hyperexcitable, hyperglutamatergic state within the CA1, subiculum and dentate gyrus subregions, and that hippocampal hyperfunction, in addition to its putative role as a biomarker of psychosis, psychosis risk, and psychosis severity, is also a novel therapeutic target amenable to diverse preventative glutamate-limiting therapeutic strategies.

Disclosure: S. Schobel: None. N. Chaudhury: None. C. Corcoran: None. N. Lewandowski: None. J. Lieberman: None. H. Moore: None. S. Small: None.

177. A Preliminary Study of Repetitive Transcranial Magnetic Stimulation for Smoking Cessation in Schizophrenia

Victoria C. Wing*, Ingrid Bacher, Zafiris J. Daskalakis, Tony George

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

Background: Rates of cigarette smoking are particularly high in persons with schizophrenia and these smokers also experience more difficulty in quitting. Moreover, standard pharmacological and

behavioural smoking cessation interventions have had limited success in this population, therefore it is imperative to identify more effective treatment options. Repetitive transcranial magnetic stimulation (rTMS) to the dorsal lateral prefrontal cortex (DLPFC) has been shown to reduce cigarette craving and consumption in smokers without a psychiatric diagnosis. And rTMS is also thought to improve cognitive performance, providing further rationale for its use in smokers with schizophrenia as smoking abstinence results in cognitive deficits in this population and poor baseline cognitive performance predicts treatment failure. The aim of this study was to determine the safety and efficacy of rTMS, in combination with transdermal nicotine patch, for the treatment of nicotine dependence in schizophrenia.

Methods: 13 participants with a DSM-IV diagnosis of schizophrenia or schizoaffective disorder participated in a 10-week randomized, double-blind, sham-controlled trial. Participants received either active (n = 6) or sham (n = 7) rTMS targeted to the DLPFC at a frequency of 20 Hz in weeks 1-4 (20 sessions; 5 treatments/week). All participants received nicotine patch from weeks 3-9 and weekly behavioural counselling. Outcome measures included smoking abstinence rates which were determined by self-report and biochemically verified by expired breath carbon monoxide; tobacco craving and withdrawal; cognitive performance; and treatment side effects.

Results: Data were analysed using an intention-to-treat approach. Active rTMS did not increase 7-day point prevalence abstinence rates at trial end point. Mixed model analyses were employed to determine if weekly outcome measures differed between active and sham groups but no significant differences in smoking measures were identified. However, there was a significant effect of treatment on craving scores obtained from the Tiffany Questionnaire of Smoking Urges (TSQU) administered immediately pre- and post-TMS treatment: in the sham group TSQU scores increased following TMS in week 1, presumably due to nicotine abstinence imposed by the 30 minute treatment session, whereas active TMS attenuated this abstinence-induced increase in craving (factor 1: $p = .03$, factor 2: $p = .04$). Active rTMS was well tolerated and did not influence schizophrenia symptomatology assessed by the PANSS ($p = .95$).

Discussion: This preliminary study found rTMS was safe to use in smokers with schizophrenia but no effects on smoking abstinence rates were identified. However, rTMS may still provide a useful adjunct to standard tobacco treatments in this hard to treat population due to its potential to reduce cigarette craving and improve cognitive function.

Disclosure: V. Wing: None. I. Bacher: None. Z. Daskalakis: None. T. George: None.

178. Gene Networks are Tightly Regulated During Early Development of Prefrontal Cortex

Michael Winger*, Tianzhang Ye, Daniel Weinberger, Joel Kleinman, Barbara Lipska

National Institute of Mental Health, Bethesda, MD

Background: Normal development of the human brain is governed by a complex interplay between the genes expressed in various brain regions, a process thought to reflect systems of gene networks, whose architecture change throughout the aging process. One example of this staged maturation is the dorsolateral prefrontal cortex (DLPFC), which continues its development through young adulthood, and is strongly implicated in autism and schizophrenia. Determining how these networks are constructed and de-constructed, and where errant network architectures beget psychiatric dysfunction, is essential for identifying causal mechanisms of developmental brain disorders.

Methods: In the present study, we obtained expression data from the (DLPFC) of 271 non-psychiatric subjects ranging in age from the second trimester of fetal life to nearly 80 years, using two-color custom-spotted arrays with the Illumina OligoSet of 49,152 70-mer probes. We limited the number of probes to 30,345 based on several criteria, including detection limit and SNP information. Lifespan

stages comprised samples of fetal age (2nd trimester of gestation), infants (0-1 years), children (1-13 years), teenagers (13-20 years), and adults in decades of 20-30, 30-40, 40-50, 50-60, and 60-80 years of age; each stage contained a minimum of 18 subjects. We characterized the composition and activity of gene networks expressed in DLPFC in terms of their specificity and functionality. Individual genes were grouped into modules via hierarchical clustering, according to correlation among expression profiles. The specificity of these gene co-expression modules was then measured directly as the average cross-correlations of expression profiles within these modules: high average correlations indicate highly similar expression patterns, suggesting a robust association of co-regulated genes, i.e. a gene network. Functionality was determined by enrichment of gene ontology (GO) terms within each module. In order to assess the time-evolution of gene networks across the lifespan, gene expression data were analyzed within non-overlapping cohorts defined according to the above age categories.

Results: We identified a large number of modules per stage using the clustering routine (mean $116 \pm SD 35$; min = 86 in children, max = 204 in 20-30 years of age, with 3304 ± 1596 genes in the largest stage module). Analysis of the within-stage average module specificity (mean of all within-module average correlation coefficients) revealed dramatic differences between early and late-life: stage averages were 0.57 ± 0.04 , 0.71 ± 0.03 , 0.70 ± 0.09 , and 0.65 ± 0.07 in early-fetal, infant, child, and teen-age stages; there were no stage averages greater than 0.42 beyond teenagers.

Preliminary integration with gene ontology data suggests modest age-dependence in stage module function: while some terms are enriched throughout the lifespan, e.g. translation, mRNA processing, protein metabolic processes; some are only enriched in narrow windows of development, e.g. structural processes (cell-substrate adhesion, microtubule-based movement, and peptide cross-linking categories enriched in fetal and infant samples) or lipid metabolism (membrane lipid biosynthesis, fatty acid metabolic processes enriched in post-natal and children). Generally, among those terms not enriched across the entire lifespan, GO enrichment was more significant in early developmental stages than in later life.

Discussion: On the evidence of higher apparent specificity in the co-expression of genes within early life, and a similar apparent trend in ontological enrichment, we conclude that the gene expression activities in the DLPFC are regulated focally during fetal life through the late teens, after which the gene networks persist in a steady state of lesser regulation.

Disclosure: M. Wininger: None. T. Ye: None. D. Weinberger: None. J. Kleinman: None. B. Lipska: None.

179. Biobehavioral Mechanisms of Topiramate and Drinking in Adolescents: Preliminary Findings

Peter Monti*, Robert Miranda, Alicia Justus, James MacKillop, Jeffery Meehan, Jennifer Tidey, Robert Swift

Brown University, Providence, RI

Background: Each year more than one million youth develop a sufficient spectrum of alcohol-related problems to warrant a diagnosis of alcohol dependence (AD), and alcohol addiction is a major impetus for substance abuse treatment admissions among teenagers. Yet despite clinical demand for effective interventions for youth, less than one-third experience sustained benefit from existing treatments; the vast majority return to drinking within 6 months post-treatment. While researchers have advanced pharmacotherapy for adults, double-blind, placebo-controlled clinical trials with adolescents are almost nonexistent. The goal of this pilot project was to gather preliminary findings on the effects of topiramate (TPM) - an anticonvulsant shown to be efficacious for treating adults - on drinking, craving, and the acute subjective effects of alcohol ingestion among adolescents.

Methods: Non-treatment seeking adolescent heavy drinkers ($n=13$; mean age = 19, $SD=1.1$) were enrolled in this pilot project. The sample was approximately half female (56%) and 78% White (22%

Black); ethnically, 33% of the sample was Hispanic. Participants were randomized to receive TPM (escalating doses up to 200 mg/day) or placebo for 5-weeks. To assess craving and subjective effects of alcohol in participants' natural environments, youth completed daily ecological momentary assessment (EMA) for the entire 5-week period and then participated in our standardized *in vivo* alcohol cue reactivity assessment while at the target dose; youth were stabilized on 200 mg/day of TPM for two weeks following a 3-week titration period.

Results: Of the 13 adolescents enrolled, 3 withdrew prior to taking medication and one participant randomized to TPM was withdrawn from the study in week 2 due to weight loss. The remaining 9 adolescents completed the 5-week protocol (TPM = 4, placebo = 5). At baseline, youth reported drinking on more than half of the previous 90 days (TPM = 59%, placebo = 64%). The average number of standard drinks consumed on days when alcohol was used indicated that participants were heavy drinkers (TPM = 6.7 [$SD=1.2$]; placebo = 8.3 [$SD=3.1$]). Medication compliance was high across the trial. TPM was well tolerated by youth; there were no serious adverse events and, aside from weight loss by one participant, no significant side effects were reported. In addition, adolescents were highly compliant with the EMA protocol. Overall, youth completed 90% of the nearly 1,600 random prompt assessments delivered on the electronic device during the study and this did not abate over the trial. Across the trial, youth entered an average of 8.6 comprehensive drink assessments per week. Finally, preliminary data showed that youth assigned to TPM had steady reductions in the number of drinking days per week (weeks 1-5: mean = 3.8, 3.3, 3, 2.7, & 2 drinks per week, respectively). And this pattern was not observed in the placebo group (weeks 1-5: mean = 4, 4.3, 3.7, 3.1, & 3.5 drinks per week, respectively). Moreover, adolescents in the TPM condition showed reductions in the number of alcoholic beverages consumed on drinking days across the trial, while youth on placebo continued to drink at baseline levels. In the lab, youth reported substantial increases in craving following exposure to alcohol cues, with a medium magnitude effect size ($f=.27$); the sample was too small, however, to test effects of TPM on cue-elicited craving.

Discussion: This pilot project supports the utility of a comprehensive yet efficient paradigm for studying drinking and mechanisms of pharmacotherapy action in youth. Notably, these data indicate that youth are more compliant with the EMA protocol than adults in our studies. In addition, this preliminary evidence suggests that TPM reduces alcohol use in youth. Although this pilot was too small to examine whether TPM blunts craving in the laboratory, on the whole these data support the tolerability and promise of TPM for use with adolescents and provide additional evidence that youth experience measurable increases in craving when faced with alcohol-associated cues. Although our findings are promising, compelling evidence indicates that the safety and efficacy of medication use with adolescents cannot be inferred from adult data. As such, these findings suggest a large-scale clinical trial of TPM among adolescents is warranted. Research supported by NIAAA (AA007850; PI: P. Monti).

Disclosure: P. Monti: None. R. Miranda: None. A. Justus: None. J. MacKillop: Part 1; Pfizer, Inc.. Part 4; Pfizer Independent Competitive Grants Program. J. Meehan: None. J. Tidey: None. R. Swift: None.

180. The Metabolic Profile of Iloperidone Compared to Ziprasidone in a Short-Term Placebo-Controlled Clinical Trial and Open-Label 25 Week Extension

Henry Nasrallah*, Peter Weiden, Leslie Citrome, Marla Hochfeld, Xiangyi Meng

University of Cincinnati, Cincinnati, OH

Background: The atypical antipsychotic iloperidone (ILO), a mixed D₂/5-HT₂ receptor antagonist, is indicated for the acute treatment of schizophrenia in adults. We present the first detailed analysis of ILO's metabolic profile, including the metabolic syndrome risk factors (fasting glucose, triglyceride [TG], high-density lipoprotein cholesterol [HDL-C], waist circumference, and blood pressure [BP]) and other

related parameters such as weight, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and glycosylated hemoglobin (HbA1C).

Methods: Data were obtained from 2 related studies: a 4-week, randomized, double-blind (DB), controlled clinical trial of ILO 24 mg/day (n = 300), placebo (PBO; n = 147) and ziprasidone (ZIP) 24 mg/day (n = 150, used as an active control) in schizophrenia as well as a subsequent open-label extension (OLE) phase in which eligible patients received ILO (a majority of patients [n = 173] received ILO at the high-dose level [24 mg/day]) for 25 weeks. Metabolic parameters (obtained from fasting blood samples during the 4-week study and random samples during the 25-week OLE) and BP were evaluated. Data are reported separately for all patients in the DB study with week 4 data and for patients who completed the OLE.

Results: Results reported as mean (standard deviation) unless otherwise noted. Glucose and HbA1C Baseline glucose levels were: ILO, 94.8 (14.4); ZIP, 95.7 (16.6); and PBO, 95.7 (13.2) mg/dL. Changes from baseline to week 4 were: ILO, 6.9 (28.4); ZIP, 5.4 (29.9); and PBO, 0.0 (18.8) mg/dL. After the OLE, glucose levels with ILO were essentially unchanged from initial DB baseline (1.3 [17.6] mg/dL). HbA1C levels \geq 15% above normal were observed in 1.9%, 1.5%, and 1.5% of ILO, ZIP, and PBO patients, respectively, in the 4-week study, and in 2 ILO patients (1.4%) in the OLE. TG Baseline TG levels were: ILO, 169.0 (112.9); ZIP, 154.3 (108.7); and PBO, 143.0 (78.0) mg/dL. Changes from baseline to week 4 were: ILO, -4.2 (80.9); ZIP, -10.9 (86.0); and PBO, 12.0 (116.4) mg/dL. After the OLE, TG levels were reduced by -14.8 (90.6) mg/dL from DB baseline. Total Cholesterol Baseline TC levels were: ILO, 190.7 (44.6); ZIP, 186.3 (39.4); and PBO, 186.4 (42.9) mg/dL. Changes from baseline to week 4 were: ILO, 10.2 (31.7); ZIP, 0.7 (31.5); and PBO, -1.4 (34.4) mg/dL. After the OLE, TC was reduced by -5.4 (26.2) mg/dL from DB baseline. LDL-C Baseline LDL-C levels were: ILO, 109.2 (37.8); ZIP, 105.0 (34.1); and PBO, 108.7 (36.5) mg/dL. Changes from baseline to week 4 were: ILO, 10.7 (30.1); ZIP, 2.0 (26.4); and PBO, 0.4 (25.6) mg/dL. After the OLE, LDL-C was reduced by -4.0 (23.3) mg/dL from DB baseline. HDL-C Baseline HDL-C levels were: ILO, 49.2 (17.5); ZIP, 51.2 (17.3); and PBO, 48.1 (13.1) mg/dL. HDL-C changes from baseline to week 4 were -0.4 (13.4), -1.0 (10.7), and -4.0 (8.3) mg/dL with ILO, ZIP, and PBO, respectively. After the additional 25 weeks of ILO treatment, HDL was essentially unchanged from DB baseline (-0.1 [11.6] mg/dL). Weight Baseline weights were: ILO, 83.2 (17.8); ZIP, 79.9 (18.5); and PBO, 81.2 (20.0) kg. Changes in weight from baseline to week 4 were 3.5 (3.5), 1.4 (2.8), and 1.0 (2.6) kg with ILO, ZIP, and PBO, respectively. During the OLE, weight change from DB baseline was 2.4 (5.9) kg. Weight gain \geq 7% occurred in 21.3%, 7.4%, and 3.4% of patients receiving ILO, ZIP, and PBO, respectively during the 4-week study. During the 25-week OLE, 37.6% of patients receiving ILO had experienced weight gain \geq 7%. Waist circumference Baseline waist circumferences were similar in the 3 groups: ILO, 94.5 (13.6); ZIP, 92.5 (13.4); and PBO, 94.1 (15.0) cm. Changes from baseline to week 4 were observed among the 3 groups: ILO, 2.5 (6.0); ZIP, 1.6 (5.2); and PBO, 1.4 (5.2) cm. BP Mean baseline systolic/diastolic BP values were: ILO, 121/76; ZIP, 122/76; and PBO, 120/78 mmHg. Changes in supine systolic BP from baseline to week 4 were: ILO, -0.7 (14.1); ZIP, 0.7 (11.5); and PBO, 2.4 (13.6) mmHg. Changes in supine diastolic BP from baseline to week 4 were: ILO, -1.9 (9.3); ZIP, 1.2 (10.6); and PBO, -0.1 (9.2) mmHg. At the end of the OLE, changes in supine systolic/diastolic BP were -5.2 (13.9) and -2.7 (10.2) mmHg, respectively.

Discussion: Data from patients who participated in the 2 studies for up to 29 weeks of ILO treatment suggest that ILO does not have the propensity to increase the development of the metabolic syndrome. One-third of patients experienced clinically notable weight gain. Mean weight changes suggest that weight gain while on iloperidone treatment stabilizes over time.

Disclosure: H. Nasrallah: Part 1; Abbott, AstraZeneca, Novartis, MerckAdvisory Boards/Consultant, Speaker Bureau, Forest, GSK,

Janssen, Otsuka, Pfizer, Roche, Sanofi-Aventis, Shire - Research Grant. Part 4; Forest, GSK, Janssen, Otsuka, Pfizer, Roche, Sanofi-Aventis, Shire - Research Grant. P. Weiden: Part 1; AstraZeneca, Bristol-Myers Squibb/Otsuka America Pharmaceutical, Eli Lilly, Forest, Ortho-McNeil Janssen, Merck, Novartis, Pfizer, Takeda, Vanda, Wyeth. Part 4; NIMH, McNeil, Janssen. L. Citrome: Part 1; AstraZeneca, Azur, Eli Lilly, GSK, Janssen, Merck, Novartis, Pfizer. Part 4; AstraZeneca, Eli Lilly, Janssen, Pfizer. M. Hochfeld: Part 5; Novartis Pharmaceutical Corporation, East Hanover. X. Meng: Novartis Pharmaceutical Corporation, East Hanover.

181. The Noradrenergic α_{1A} Receptor Antagonist Doxazosin Attenuates the Subjective Effects of Cocaine in Non-Treatment-Seeking, Cocaine-Dependent Volunteers

Thomas Newton*, Colin Haile, Richard De La Garza, Thomas Kosten
Baylor College of Medicine, Houston, TX, Baylor College of Medicine
Michael E. DeBakey VA, Houston, TX

Background: The focus of treatment development research for cocaine dependence has been on agents that alter central dopamine (DA), GABA, or glutamate signaling. More recently, genetic and pharmacological evidence has confirmed that noradrenergic mechanisms contribute to the effects of cocaine and other addictive drugs. For example, animals lacking the noradrenergic α_1 receptor ($\alpha_{1A}R$) are insensitive to the locomotor activating effects of stimulants, and treatment with the $\alpha_{1A}R$ antagonist prazosin blocks both cocaine-induced locomotor activation and cocaine-induced reinstatement of extinguished cocaine seeking behavior. The prototypical $\alpha_{1A}R$ antagonist prazosin has an elimination half-life of 2 to 3 hours in humans, requiring frequent dosing. This is problematic due to poor adherence associated with frequent dosing. Doxazosin is a newer $\alpha_{1A}R$ antagonist with an elimination half-life of 22 hours in humans, allowing once-daily dosing. After confirming in rodents that doxazosin penetrated the blood-brain-barrier and produced effects similar to those of prazosin in an acute locomotor activation study, we tested the effects of doxazosin in non-treatment-seeking, cocaine-dependent volunteers.

Methods: The protocol was approved by our IRB and all participants gave signed informed consent. Participants were non-treatment-seeking, cocaine-dependent volunteers. We used a within-subjects design in which participants were assessed during treatment with doxazosin and placebo, with study episodes separated by at least 2 weeks. Doxazosin tablets were over-encapsulated and placebo capsules were used to maintain the blind. Doxazosin treatment was started at 1 mg/day and increased over 9 days to 4 mg/day. Dosing occurred at 8am daily. Placebo was dosed identically. Orthostatic blood pressure measurements were taken at frequent intervals after study medication dosing. Effects of cocaine were tested after 3 days of treatment with 4 mg/day doxazosin/placebo. Cocaine (20 and 40 mg) was administered IV in that order with a dose of placebo saline randomly interspersed to maintain the blind. Dosing occurred at 1-h intervals.

Results: To date, cocaine-dependent participants treated with doxazosin (4 mg/d; N = 7, with N = 10 planned) exhibited substantial reductions in "High", "Stimulated", and "Desire Cocaine" assessed using visual-analogue scales administered prior to and following cocaine administration. Doxazosin treatment was well tolerated, and no participant experienced treatment-emergent side effects other than asymptomatic orthostatic hypotension. This occurred most prominently after treatment initiation and after dosage increases.

Discussion: These results are consistent with earlier research showing that disulfiram treatment reduces cocaine use. A newer drug with a similar mechanism of action, SYN-117 (nepicastat), also reduces the positive subjective effects of cocaine. Both disulfiram and SYN-117 inhibit dopamine- β -hydroxylase, which mediates the conversion of DA into norepinephrine (NE), thus reducing the availability of NE for release. Doxazosin directly inhibits the binding

of NE to $\alpha_{1A}R$, reducing noradrenergic signaling by that mechanism. Both approaches show promise for the treatment of cocaine dependence.

Disclosure: T. Newton: None. C. Haile: None. R. De La Garza, II: None. T. Kosten: None.

182. Prophylactic Efficacy of Fluoxetine, Escitalopram, Sertraline and Paroxetine in Unipolar Depression: Outcome After Long-Term Follow-Up

Eric Peselow*, Shannon Stepan, Magda Campbell

Maimonides Medical Center, Brooklyn, NY

Background: Unipolar major depressive disorder is a chronic, comorbid and impairing mental disorder with high lifetime prevalence. Although acute efficacy of antidepressant treatment is well established (1), the relative prophylactic efficacy of the antidepressants is yet to be fully determined. Studies in naturalistic settings are important in evaluating treatment outcomes with antidepressants, since controlled clinical trials include only minority of patients present in clinical practice (2). This study was conducted to determine the prophylactic effectiveness of 4 different types of commonly used newer generation antidepressants (fluoxetine, escitalopram, sertraline and paroxetine) in unipolar major depressive disorder with long term follow-up, and to identify predictors of outcome.

Methods: A 9-year follow up study was conducted among three eighty seven patients > or = 18 years treated at the outpatient clinic ... Patients met the DSM-IV criteria for major depressive episodes, or for depressive episode not otherwise specified. Outcome was assessed by the presence of recurrent episode of depression during the prophylaxis therapy with antidepressants. Predictor variables were: presence of previous depressive episodes, comorbid psychiatric conditions, including personality disorders and co-treatment with psychotherapy during the prophylaxis.

Results: During an average follow-up period of 34.46 mos, 23.49% of our patients remained episode free and 76.51% of patients had a relapse of depressive episode during the duration of the prophylaxis with antidepressants. Interestingly, escitalopram and fluoxetine had the highest prophylactic efficacy (36% and 33.33% of the patients remained symptom free, respectively), while paroxetine and sertraline showed poor efficacy in prevention of depressive episodes (12.8% and 21.3% of patients remained symptom free, respectively). Neither presence of co-morbid psychiatric condition nor history of previous depressive episodes was predictive of the outcome of prophylaxis. However, co-treatment with psychotherapy dramatically increased prophylactic efficacy of antidepressant treatment, 41.17% of patients undergoing psychotherapy remained episode free as compared to 18.09% patients who received only pharmacological treatment during the prophylaxis period ($p < 0.05$).

Discussion: The results of this study with its long observation period indicate that escitalopram and fluoxetine appear to have prophylaxis efficacy in the treatment of unipolar depression in a naturalistic setting. A positive response to prophylaxis was associated with co-treatment with psychotherapy. Taken together these preliminary data may help in the further definition of the range of clinical utility of these widely used antidepressants in treatment and prophylaxis of unipolar depressive disorder.

Disclosure: E. Peselow: None. S. Stepan: None. M. Campbell: None.

183. Bipolar Disorder in U.S. versus Europe

Robert Post*, David Luckenbaugh, Heinz Grunze, Willem Nolen, Paul Keck, Gabriele Leverich, Trisha Suppes, Susan McElroy, Ralph Kupka, Michael Rowe, Lori Altshuler

Bipolar Collaborative Network, Bethesda, MD

Background: Controversy persists about the high incidence of childhood onset bipolar illness in the U.S. compared with some other

non North American countries. We wished to explore these differences and those related to subsequent course of illness and treatment response in the U.S. vs. two European countries.

Methods: Our bipolar collaborative network had 4 sites in the U.S. (Los Angeles, Dallas, Cincinnati, Bethesda) and 3 in Europe (E=Utrecht, The Netherlands and Freiburg and Munich, Germany). 525 outpatients average age 42 with bipolar disorder gave informed consent, completed a questionnaire on demographics and course of illness, were rated prospectively on a daily basis on the clinical version of the NIMH-LCM for at least one year, and compared on illness characteristics, treatment strategies, and sustained long-term responses (>6 months) in those studied and treated naturalistically in the U.S. vs. Europe.

Results: Patients from the U.S. sites, compared to those from Europe, had an earlier onset of illness (22% in U.S. prior to age 13 vs. 2% in Europe), more psychosocial adversity (physical or sexual abuse), and greater parental loading for bipolar illness in one parent (20.5% in U.S. vs. 9.8% in Europe) or any mood disorder in both parents reflecting assortative mating (14.6% in U.S. vs. 3.8% in Europe). They also had more anxiety and substance abuse comorbidity, higher incidence of more than 20 lifetime episodes and of rapid cycling in the year prior to network entry. Those from the U.S. were less "Well on Network entry" (12.3%) than from Europe (29.5%), more long-term Nonresponders in prospective follow up (U.S. 51.8% vs. Europe 31.31%) as opposed to long-term Responders who were very much or much improved for at least 6 months. The Responders began their improvement after an average of 1.5 years in the Network and were treated with an average of 3 medications after 2 others were tried and discontinued. Nonresponders were also treated an average of 3 medications at any one time and were exposed to an average of 7 unsuccessful medication trials. Patients from the U.S. were treated more often with valproate (VPA) (68% in U.S. vs. 55.8% in Europe), antidepressants (ADs) (79.3% in U.S. vs. 63.6% in Europe), and atypical antipsychotics (AAs) (50.7% in U.S. vs. 32.6% in Europe) while those from Europe were treated more often with lithium (81.4% in Europe vs. 55.3% in U.S.), typical antipsychotics, and benzodiazepines. However, in Europe compared to the U.S. there were higher long-term treatment success rates for lithium (49.5% in Europe vs. 28.9% in the U.S.) and AAs (31.0% in Europe vs. 15.8% in the U.S.) with a nonsignificant trend in the same direction for VPA (37.5% in Europe vs. 27.5% in the U.S.).

Discussion: To the extent that our Network patients were representative, U.S. patients had an early onset and a more pernicious course of illness as both self-reported retrospectively and clinician-rated prospectively. The reasons for the preteen onset being 11 fold more common in the U.S. remains to be further explored, but includes our findings of two known risk factors for early onset: 1) an increased familial loading for affective illness; and 2) a higher incidence of severe psychosocial adversity (47.6% in the U.S. vs. 29.0% in Europe). Early age of onset illness was associated with longer delay to first treatment of depression or mania ($r = -.46$, $p < .001$) and averaged more than 15 years in those with preteen onsets and 10 years with adolescent onsets. Independently, longer delay to first treatment was associated with more prospective severity and time depressed, and more episodes and ultradian cycling and fewer days euthymic. These data suggest the potential importance of earlier treatment intervention in the 61% (our study) to 66% (Perlis et al., 2004, STEP-BD) of U.S. patients with childhood or adolescent onset bipolar disorder (and perhaps more use of lithium) in hopes of yielding a more benign outcome for early onset bipolar disorder.

Disclosure: R. Post: Part 1; Astra Zeneca, Bristol-Myers Squibb, GlaxoSmithKline. Part 2; Astra Zeneca, Bristol-Myers Squibb. Part 3; Astra Zeneca, Bristol-Myers Squibb. D. Luckenbaugh: None.

H. Grunze: Part 1; Astra Zeneca, BMS, Eli Lilly, GlaxoSmithKline, Organon, Pfizer Inc., Sanofi-Aventis, Servier, UBC, Cephalon, Gedeon Richter, Merck, Sepracor. Part 2; Astra Zeneca, BMS, UBC. Part 3; Astra Zeneca, BMS, UBC. W. Nolen: Part 1; Astra Zeneca, Pfizer, Servier, Wyeth. Part 4; Netherlands Organisation for Health Research and Development, European Union, Stanley Medical Research

Institute, Astra Zeneca, Eli Lilly, GlaxoSmithKline, Wyeth. **P. Keck:** Part 1; Alkermes, Astra Zeneca, Cephalon, GlaxoSmithKline, Eli Lilly, Epi-Q Inc., Jazz Pharmaceuticals, Marriott Foundation, NIMH, Orexigen, Pfizer, Inc., Shire. Part 3; Sepracor, Inc., Medco, GlaxoSmithKline, Schering Plough, BMS, Pfizer, QuantiaMD. **G. Leverich:** None. **T. Suppes:** Part 1; Astra Zeneca, Abbott Laboratories, JDS Pharmaceuticals, LLC, NIMH, Stanley Medical Research Institute, Orexin Therapeutics, Medscape, CME Outfitters, Jones and Bartlett, Wolters Kluwer, Pharma Solutions, Pfizer. Part 4; Astra Zeneca, Abbott Laboratories, NIMH, JDS Pharmaceuticals, LLC, Stanley Medical Research Institute. **S. McElroy:** Part 1; Alkermes, Eli Lilly, Schering-Plough, Sepracor, BMS, GSK, Pfizer. Part 4; Alkermes, AstraZeneca, Cephalon, Eli Lilly, Forest, Jazz, Orexigen, Pfizer, Shire, Takeda. **R. Kupka:** Part 1; Eli Lilly, Astra Zeneca. **M. Rowe:** None. **L. Altshuler:** Abbott, Forest, GSK. Part 4; Abbott.

184. Influence of Catechol-O-Methyl Transferase (COMT) Val158

Met Genotype and COMT Enzyme Inhibitor Tolcapone on

Delta-9-Tetrahydrocannabinol Effects in Humans

Mohini Ranganathan*, Jacqueline Elander, Michelle Carbuto, Richard Sewell, Ashley Williams, Joel Gelernter, Deepak C. D'Souza

Yale University, New Haven, CT

Background: A functional polymorphism of the catechol-o-methyl transferase (COMT) gene moderates the influence of adolescent cannabis exposure on developing schizophrenia in adulthood. Laboratory and experience sampling studies suggest that COMT polymorphisms also moderate some of the acute effects of delta-9-tetrahydrocannabinol (THC) and cannabis, respectively. Homozygotes for the functional polymorphism in the COMT gene (met158val)[Val-Val] have a 40% higher COMT enzyme activity resulting in decreased dopamine (DA) availability in the prefrontal cortex (PFC) and associated worse PFC cognitive performance, as compared to the homozygotes val158met[Met-Met]. Furthermore, Val homozygotes show increased tyrosine hydroxylase gene expression and consequent DA, especially in neuronal populations relevant to psychosis.

Methods: We characterized the 1) effects of COMT genotype alone and 2) and the interactive effects of the COMT enzyme inhibitor Tolcapone and COMT genotype, on the psychotomimetic and amnestic effects of intravenous THC in a double-blind, randomized, placebo-controlled laboratory study of healthy human subjects.

Results: In this ongoing study, COMT genotype alone does not influence the psychotomimetic or amnestic response to THC. While Val homozygotes do not have a greater psychotomimetic response to THC alone relative to Met homozygotes, with tolcapone pretreatment they have greater THC-induced psychotomimetic effects as measured by the PANSS positive symptom subscale.

Discussion: While there are no differences in THC-induced immediate recall deficits between groups, tolcapone reduced the THC-induced recall deficits in Met homozygotes. The implications of these preliminary findings will be discussed.

Disclosure: M. Ranganathan: None. J. Elander: None. M. Carbuto: None. R. Sewell: None. A. Williams: None. J. Gelernter: None. D. D'Souza: None.

185. Mechanisms of Action of Quetiapine for Alcohol Dependence:

A Pilot Study

Lara Ray*, Pauline Chin, Andia Heydari, Robert Swift, Karen Miotto

UCLA, Los Angeles, CA

Background: The available treatments for alcoholism are only modestly effective and patients vary widely in their treatment response. Quetiapine, an atypical antipsychotic medication with antagonist activity at D₁ and D₂, 5-HT_{1A} and 5-HT_{2A}, H₁ and α₁ and

α₂ receptors, was shown to promote abstinence, reduce drinking days, and reduce heavy drinking days in a 12-week double-blind placebo-controlled trial. Thus, quetiapine represents one of the novel and promising pharmacotherapies for the treatment of alcoholism. However, the mechanisms of action of quetiapine for alcoholism are poorly understood.

Methods: This pilot study randomized 20 non-treatment seeking alcohol dependent individuals to one of the following conditions in a double-blind, placebo-controlled design: (1) quetiapine (400 mg/day); or (2) matched placebo. Participants were on the target medication dose (or matched placebo) for 4 weeks during which they completed weekly assessments of drinking, sleep, mood, and anxiety. All participants completed two counterbalanced intravenous placebo-alcohol administration sessions as well as a cue-reactivity assessment.

Results: A total of 20 participants were randomized, 5 dropped out of the study, 11 have completed the protocol, and 4 are currently enrolled. Preliminary analysis of the first 8 completers revealed a trend-level medication × alcohol effect, such that quetiapine reduced cue-induced craving for alcohol post alcohol administration (Breath Alcohol Concentration = 0.06) but did not reduce cue-induced craving post saline infusion [$F(1,7) = 4.27$; $p = .08$]. Moreover, participants receiving quetiapine reported lower levels of alcohol craving (measured by the Penn Alcohol Craving Scale; PACS), than placebo-treated participants across the four weeks on the target dose [$F(1,7) = 5.58$, $p = .056$]. Analyses indicated that quetiapine reduced sleep disturbance (measured by the Pittsburgh Sleep Quality Inventory; PSQI) across the four-week period on the target dose, as compared to placebo [$F(1,7) = 5.09$; $p = .06$].

Discussion: Results from this pilot study contribute critical new information about mechanisms of response to quetiapine for alcoholism, which in turn can inform larger scale studies and ultimately, clinical practice.

Disclosure: L. Ray: Part 4; Pfizer. P. Chin: None. A. Heydari: None. R. Swift: None. K. Miotto: None.

186. Galantamine Improves Sustained Attention in Chronic Cocaine Users

Mehmet Sofuoğlu*, Andrew Waters, James Poling, Kathleen Carroll

Yale University, West Haven, CT

Background: Chronic cocaine users are known to have cognitive deficits that are predictive of poor treatment response. Whether these deficits improve with medications targeting specific cognitive functions has not been examined in previous studies. The goal of this study was to evaluate galantamine's efficacy on selected cognitive outcomes, including measures of sustained attention, response inhibition, and attentional bias in recently abstinent cocaine users. Galantamine, a reversible and competitive inhibitor of acetylcholinesterase, is used clinically in the treatment of Alzheimer's dementia. We hypothesized that galantamine would be well-tolerated and improve performance in cognitive functions including attention, response inhibition, and attentional bias in cocaine users.

Methods: In a randomized, double-blind, parallel-group study, 34 participants were randomized to galantamine (8 mg/day) or placebo treatment for 10 days. Cognitive and self-report mood measures were obtained at baseline and on days 5 and 10 after the initiation of treatment. Cognitive Performance was assessed with 3 tests from the Cambridge Neurological Test Automated Battery (CANTAB): the Rapid Visual Information Processing test (RVIP), the Paired Associates Learning (PAL), and the Pattern Recognition Memory (PRM). The RVIP is widely used as a measure of sustained attention with a small working memory component. While PAL is sensitive to visual memory and learning, PRM is sensitive to visual recognition and memory. In addition, the Sustained Attention to Response Task (SART) and a modified Stroop task (cocaine-Stroop) were also administered. The SART is a Go/NoGo task which assesses the ability

to activate (Go) or inhibit (NoGo) responses. The cocaine-Stroop task assesses attention capture (attentional bias) by cocaine cues.

Results: Of the 34 participants randomized to treatment, 28 (9 female and 19 male) completed the study. Most of the participants were currently in treatment ($n=17$) and had no other drug use, except cigarette smoking ($n=21$) and marijuana use ($n=4$). Galantamine treatment, compared to placebo, improved the reaction time (RT), $F(2,50)=8.6$, $p<0.01$, detection sensitivity (A'), $F(2,50)=4.9$, $p<0.03$, number of hits, $F(2,50)=4.2$, $p<0.04$, and number of correct rejections, $F(2,50)=5.6$, $p<0.02$, on the Rapid Visual Information Processing (RVIP) task. On the cocaine-Stroop task, there was also a significant treatment-by-time interaction on overall RTs (but not on the Stroop or carry-over effect). There were no baseline differences in any of these cognitive measures. No other significant treatment-by-time interactions were observed.

Discussion: The selective improvement of the RVIP task performance by galantamine treatment in abstinent cocaine users is consistent with the well-established role of the brain cholinergic system in attentional processes. Medications that enhance the cholinergic transmission, like cholinesterase inhibitors, improve performance in the RVIP and similar tasks in patients with compromised cognitive function including Alzheimer's dementia or mild cognitive impairment. In contrast to the RVIP findings, galantamine treatment did not improve performance in other tasks tapping response inhibition, attentional bias, and visuospatial learning/memory functions in abstinent cocaine users. These results demonstrate that medications can enhance cognitive function (e.g. sustained attention) in abstinent cocaine users. Cognitive enhancement strategies may especially be important early in the treatment, when stimulant users have greater cognitive impairments following cessation of stimulant use. For example, galantamine may augment the efficacy of behavioral treatments in cocaine users, by improving their ability to learn, remember, and implement new skills and coping strategies. The potential efficacy of galantamine as a treatment for cocaine abuse needs to be further evaluated in clinical trials.

Disclosure: M. Sofuooglu: None. A. Waters: None. J. Poling: None. K. Carroll: None.

187. A Double-Blind, Placebo-Controlled Trial of Quetiapine for the Treatment of Mixed Hypomania in Bipolar II Patients

Trisha Suppes*, Terence Ketter

VA Palo Alto Health Care System & Department of Psychiatry and Behavioral Sciences Stanford University School of Medicine, Palo Alto, CA

Background: While the lifetime prevalence of DSM-IV defined bipolar II disorder (BDII) is estimated at 1.1% or more,¹ there are limited data to guide treatment of BDII, and virtually no studies on treatment or characteristics of mixed hypomania in BDII. Patients with BDII experience similar levels of psychosocial disability as those with BDI.²⁻³ Recent work demonstrates that depressive symptoms during hypomania are common, occurring in 76% of visits where hypomania was noted for patients with BDII.⁴

Methods: The study was a two-site, randomized, placebo-controlled, double-blind, 8-week investigation of adjunctive quetiapine (QTP) versus placebo (PBO) for the treatment of patients diagnosed with BDII experiencing mixed hypomania defined as scores of >12 on the YMRS and >15 on the MADRS at two consecutive visits. Primary outcomes included reduction of symptoms on the YMRS (hypomania) and MADRS (depression). QTP or PBO was added to stable psychotropic regimens; and adjunctive use of lorazepam was allowed during the first two weeks.

Results: Fifty-five patients with BDII were randomized to receive either QTP ($n=30$) or PBO ($n=25$). At one site more females were enrolled (87% vs. 64%, $p=.049$), and the PBO group reported more lifetime depressive episodes (4.2 vs. 3.6; $p=.03$). No other significant differences in demographics or clinical characteristics were noted

between sites or treatment groups. Thirty patients (54.5%) were not prescribed other psychotropics during study duration and received monotherapy QTP versus PBO; 17 were prescribed one psychotropic, 7 were prescribed two, and one individual was prescribed 3 psychotropics. Twenty-seven patients completed all eight weeks (QTP = 16; PBO = 11). Mixed-model random regression revealed a significant interaction effect (group x time) for change in CGI-Overall Severity ($F=10.12$ ($df=1$); $p<.01$) and total MADRS (depression) score ($F=6.93$ ($df=1$); $p<.01$), with significant differences favoring the QTP group. For total YMRS (hypomania) score, there were no significant differences between treatment groups ($F=3.68$ ($df=1$); $p=.07$). Secondary outcomes included functioning (GAF; $F=4.40$ ($df=1$); $p=.045$) and tolerability of QTP versus PBO. The most commonly reported side effect of at least moderate intensity was sedation, reported by 16 in the QTP group (PBO = 4; $\chi^2=8.21$ ($df=1$); $p=.05$ participants in the QTP group).

Discussion: Adjunctive quetiapine was associated with reduction in depression and hypomania and increase in function in patients with bipolar II experiencing mixed hypomania. Further analyses and characteristics of mixed hypomania in BDII will be presented.

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- Source of Funding - This research was supported by AstraZeneca Pharmaceuticals (ISS 0333).

Disclosure: T. Suppes: Part 1; Abbott Laboratories, AstraZeneca, JDS Pharmaceuticals, LLC, National Institute of Mental Health, Stanley Medical Research Institute, Pfizer Inc., Orexin Therapeutics, CME Outfitters, Medscape, Jones and Bartlett (formerly Compact Clinicals), Wolters Kluwer, Pharma Solutions (CNS Drug Supplement). Part 4; Abbott Laboratories, AstraZeneca, JDS Pharmaceuticals, LLC, Natinoal Institute of Mental Health, Stanley Medical Research Institute, Pfizer Inc. T. Ketter: Part 1; Abbott Laboratories, AstraZeneca Pharmaceuticals LP, Bristol-Myers Squibb Company, Cephalon Inc., Eli Lilly and Company, GlaxoSmithKline, Pfizer Inc., Sepracor, Inc., Astellas Pharmaceuticals, Dainippon Sumitomo Pharmaceuticals, Forest Labs, Janssen Pharmaceutica Products, LP, Merck & Co., Inc., XenoPort, Inc., Johnson & Johnson (Nzeera Ketter, MD, Spouse). Part 2; GlaxoSmithKline, Johnson & Johnson (Nzeera Ketter, MD, Spouse). Part 3; Johnson & Johnson (Nzeera Ketter, MD, Spouse). Part 4; AstraZeneca, Eli Lilly and Company, Quintiles, Inc. (Prime Sponsor: Sepracor Inc.). Part 5; Johnson & Johnson (Nzeera Ketter, MD, Spouse).

188. The Relationship between the Stimulant Effects of Alcohol and Alcohol Craving

Robert Swift*, George Kenna, Lorenzo Leggio, John McGahey, William Zywiak, Andrea Grenga, Junghyun Kim, Nancy Ciminelli

Brown University, Providence, RI

Background: The Psychomotor Stimulant theory of addiction posits that the dependence-producing properties of alcohol and drugs of abuse are associated with the positively reinforcing and stimulant properties of the substances. Alcohol is simultaneously stimulating and sedative, producing greater stimulation at lower blood alcohol concentrations (BACs) and rising BACs and producing greater

sedation at higher BACs and falling BACs. Effective pharmacotherapies for alcohol dependence that reduce stimulation, including naltrexone and dopamine antagonists (e.g. olanzapine, aripiprazole) also reduce craving for alcohol. However, pharmacotherapies that do not attenuate stimulation, such as acamprosate, do not appear to attenuate craving and topiramate may attenuate stimulation without affecting craving. Two human laboratory alcohol administration studies provided an opportunity to determine the relationship between alcohol-induced stimulation, subjective craving and alcohol consumption in alcohol dependent and non-dependent individuals.

Methods: Alcohol-dependent and non-alcohol dependent individuals were recruited and tested as follows: Alcohol-dependent participants, who were non-treatment seeking and medically screened received double-blind treatment with placebo, topiramate (max. 200 mg), aripiprazole (max. 15 mg), or topiramate + aripiprazole, over a 6 week period. At the end of treatment, participants had a laboratory alcohol self administration study during which they consumed a priming dose of oral alcohol to produce a BAC 30 mg/dl. After 1 hour, they were allowed to consume up to 8 "drinks" of an alcoholic beverage or they could receive an alternate reinforcer of \$3 in cash for each drink not consumed. Throughout the session, alcohol-induced stimulation and sedation were measured with the Biphasic Alcohol Effects Scale (BAES) and craving was measured with the Alcohol Urge Questionnaire (AUQ). In a separate experiment, medically screened non-alcohol dependent participants were administered oral alcohol on two different occasions: to achieve a peak BAC of 40 mg/dL and 80 mg/dL, respectively. Alcohol-induced stimulation and sedation were measured with the BAES and craving was measured with the AUQ throughout the session. All participants were genotyped for OPRM1 (asp/asn) and DRD4 (L/S).

Results: In the alcohol dependent participants, the AUQ score and the Stimulation Scale of the BAES were significantly correlated ($r = .36$, $p = 0.03$, $n = 37$) 30 minutes after the priming drink was administered, when stimulation was the greatest. However, neither BAES stimulation scale scores nor the AUQ scores were correlated with the amount of alcohol self-administered in the laboratory. The non-alcohol dependent subjects showed correlations of similar magnitude between the AUQ score and the Stimulation Scale of the BAES ($r = .36$, $p = 0.02$ for the lower 40 mg/dl peak BAC, $n = 40$). The correlation between AUQ and Stimulation Scale of the BAES for the higher 80 mg/dL peak BAC was not significant ($r = .22$, $p = 0.24$, $n = 30$).

Discussion: Subjective stimulation as measured by the BAES and alcohol craving as measured by the AUQ were positively and significantly correlated in these two samples of non-treatment seeking alcoholics and non-alcoholic dependent drinkers, particularly at low BACs on the ascending limb, where stimulation is greater. However, the correlations are low, suggesting that there is variability between individuals. Surprisingly, neither stimulation nor craving was correlated with alcohol self-administration in the alcohol-dependent participants. Additional analyses will explore the effects of genetics and other individual differences on the relationship between craving and stimulation. Supported by NIAAA grant R01AA015753.

Disclosure: R. Swift: Part 1; Alkermes, Inc., D.B. Pharma, Eli Lilly. G. Kenna: None. L. Leggio: None. J. McGeary: None. W. Zywiak: None. A. Grenga: None. J. Kim: None. N. Ciminelli: None.

189. Functional Signatures of Neuroactive Drug Action Identified by Brief Resting-State MEG Scans

Todd Verdoorn*, Rajasimhan Rajagovindan, Jeffrey Lewine, Concetta Forchetti

Alexian Brothers Neuroscience Research Institute, Oak Grove Village, IL. Orasi Medical, Inc., Minneapolis, MN

Background: Successful development of CNS drugs often requires an accurate early assessment of pharmacodynamics, dose-response and drug mechanism. It is well established that many neuroactive drugs

alter human brain function in ways that readily can be detected by standard electrophysiological methods such as electroencephalography (EEG) or magnetoencephalography (MEG). However, excessive variability and lack of standardization often limits the utility of EEG for supporting CNS drug development projects. We report here the results of a recent MEG study designed to investigate the application of brief resting-state MEG scans for evaluating the acute effect of neuroactive drugs and thereby supporting efforts to characterize experimental compounds in early development.

Methods: A randomized, placebo-controlled, crossover study was conducted to investigate the effect of modafinil (100 mg, p.o.), methylphenidate (20 mg, p.o.), and lorazepam (1 mg, p.o.) on brain activity in 15 healthy male volunteers. Subjects were evaluated with a single test of cognitive function as well as MEG and EEG at baseline and 2, 4, and 6 hours after a single, oral dose of one of three drugs or placebo. Subjects were given one of the four treatments on each of 4 study days, which were separated by one week. One minute long resting-state profiles were analyzed separately over 8 predefined groups of sensors using a regional variant of the Synchronous Neural Interaction test (SNI) and a standard frequency-domain approach, to detect and quantify drug-induced changes in brain activity.

Results: Lorazepam decreased global alpha band (8-13 Hz, $p < 0.001$) spectral power and increased delta activity (1-4 Hz, $p < 0.002$) compared to baseline. It also increased the level of correlated brain activity measured by the SNI test, largely in frontal regions ($p < 0.008$). Methylphenidate increased alpha activity over parietal, temporal and central regions ($p < 0.02$) and decreased SNI between left frontal and left temporal areas ($p < 0.007$). Modafinil increased alpha activity only in the parietal region ($p < 0.04$). Notably, the patterns of the sedative drug (lorazepam) were generally opposite to those induced by the mild stimulants (modafinil and methylphenidate), especially with respect to the level of correlated activity measured by the SNI test. Direct comparison of the MEG and EEG scans (collected simultaneously) showed that MEG was less variable and more sensitive to changes in higher frequency bands. Drug-induced changes in cognitive function were less robust than either electrophysiological endpoint and were not statistically significant.

Discussion: These observations demonstrate that brief resting-state MEG scans detect changes in brain function more readily than simple tests of cognitive function. The drugs tested altered patterns of brain connectivity based on synchronous correlations (SNI test) as well as rhythmic activity measured by standard frequency domain methods. The results support the development of multivariate statistical models that can more thoroughly characterize the effect of neuroactive drugs based on different facets of resting-state electrophysiology data. Quantitative comparisons of novel compounds to a database of known drug signatures can provide better understanding of pharmacodynamic effect, mechanism, and therapeutic effect. The brief and simple nature of the scan tasks improves reproducibility and reliability and facilitates the recruitment, compliance and retention of study volunteers. Additional work is ongoing to characterize the actions of drugs with other mechanisms and demonstrate the ability of this technology to accurately measure dose-response and pharmacodynamic-pharmacokinetic relationships.

Disclosure: T. Verdoorn: Part 5; Orasi Medical, Inc. R. Rajagovindan: Orasi Medical, Inc. J. Lewine: None. C. Forchetti: None.

190. Clinical Predictors of Treatment Response to Divalproex and Risperidone Among Youth with Pediatric Bipolar Disorder (PBD)

Amy West*, Sally Weinstein, Mani Pavuluri

University of Illinois at Chicago, Chicago, IL

Background: Pharmacotherapy is the front-line approach for reducing symptoms of PBD; however, findings in the literature frequently indicate nonresponse rates of more than 40% (Emslie et al., 2003). Understanding the factors that differentiate bipolar youth who

respond positively to treatment versus those who do not is key to developing more effective interventions and informing future research and practice parameters. The current study examined three relevant clinical risk factors at baseline that may influence response to pharmacotherapy among children and adolescents with PBD: (1) high levels of aggression, (2) high levels of irritability, and (3) comorbid disruptive behavior disorders. Indicators of treatment response included measures of PBD symptomatology and global psychosocial functioning. In addition, we examined the interactions between clinical predictors and medication type (risperidone versus divalproex) to begin to address the question of what works under which conditions.

Methods: Data for this study were collected as part of a six-week double-blind, placebo-controlled, randomized outpatient medication treatment trial of risperidone versus divalproex for mania. Sixty-six children and adolescents (mean age = 10.9 years, SD = 3.3) with PBD were followed prospectively throughout pharmacotherapy treatment. Aggression and irritability were assessed at baseline via the Overt Aggression Scale-Aggression and Irritability subscales (OAS); mean splits were used to categorize high versus low levels of aggression and irritability. Presence of comorbid disruptive behavior disorders at baseline was assessed using the Washington University Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-K-SADS). Outcome measures of PBD symptoms included the Young Mania Rating Scale (YMRS) and the Child Depression Rating Scale-Revised (CDRS-R). Global functioning was assessed via the Child and Adolescent Functioning Assessment Scale (CAFAS). All outcome measures were administered weekly for 8 weeks of treatment to examine both the magnitude and trajectory of symptom change over time. Mixed-effects regression models were examined separately for each clinical predictor (Aggression, Irritability, Comorbidity) and each outcome measure (YMRS, CDRS-R, CAFAS). Models included interactions between time and the clinical predictor to examine differences in symptom change over time at each level of the predictor variable (high/low aggression; high/low irritability; presence/absence of comorbidity); in addition, Time x Clinical Predictor x Active Drug (risperidone v. divalproex) effects were included in each model to examine differential medication responses among the clinical predictors.

Results: Findings indicated that the clinical predictors significantly influenced treatment response. PBD youth with high levels of baseline aggression experienced a greater rate of change in depressive symptoms over time than PBD youth with low levels of aggression, across medication groups (Estimate = -1.95; SE = .99, t (339) = -1.98, p = .05). Youth with high irritability at baseline experienced faster improvement in manic symptoms for those receiving risperidone as compared to low-irritable youth (Estimate = 0.96, SE = .46, t (329) = 2.10, p = .037); high-irritability youth also showed a greater rate of change in depressive symptoms across treatment than low-irritability youth, across medication groups (Estimate = -5.81, SE = 2.53, t (329) = -2.30, p = .022). Last, youth with comorbid disruptive behavior disorders experienced a greater response to risperidone versus divalproex (Estimate = 7.17, SE = 2.78, t (356) = 2.58, p = .01). Regarding global functioning, all clinical predictors showed similar effects over time: youth with greater levels of risk (e.g., high aggression, high irritability, and comorbidity) at baseline experienced significant improvement in global functioning over time, but experienced lower levels of global functioning at the end of treatment as compared to youth with lower levels of risk.

Discussion: Results suggest that the presence of clinical risk factors at baseline among PBD youth was associated with different trajectories of treatment response (i.e., different rates of change or differential response to medications). Interestingly, higher levels of clinical risk at baseline was not associated with worse change in symptoms across treatment, but was associated with poorer global functioning outcomes. These findings speak to the complexity of PBD patients' response to pharmacological treatment and have important implications for future research and clinical practice.

Disclosure: A. West: None. S. Weinstein: None. M. Pavuluri: Part 1; Bristol Myer Squibbs, Ortho McNeil Pharmaceuticals.

191. Item Level Patterns of Response of Aripiprazole for the Acute Treatment of Pediatric Bipolar I Disorder

Eric Youngstrom*, Joan Zhao, Raymond Mankoski, Candace Andersson, Robert McQuade, Ronald Marcus, Robert Findling

University of North Carolina, Chapel Hill, NC

Background: There are three major reasons to conduct item level analyses of response data: (a) the effects of an intervention may not be uniform across domains assessed, (b) psychometrically weak items may underestimate treatment response, and (c) contradictory effects on specific symptoms may cancel each other out in the aggregate score. Each of these issues is likely to be relevant in clinical trials focused on pediatric mania that utilize the Young Mania Rating Scale (YMRS) total score as the primary outcome, since several items have shown poor psychometric properties (1, 2). Additionally, in the broader clinical context, some compounds have more effect on aggressive behavior, others on impulsivity or sleep, and interventions that address irritable mood and aggression address the presenting problems driving most clinical referrals. The present study simultaneously evaluated effect sizes on items from multiple outcome measures (including parent and subject self-report as well as clinician ratings) in a large pediatric sample receiving acute treatment for bipolar I disorder.

Methods: This was a *post-hoc* analysis of 296 subjects aged 10-17 years who participated in a 4-week double-blind randomized, controlled trial comparing 10 or 30 mg/day of aripiprazole versus placebo for the treatment of an acute manic or mixed episode associated with bipolar I disorder. The primary efficacy outcome measure for the trial was the mean change from baseline to endpoint in the clinician-completed YMRS total score. Clinicians also administered the Child Depression Rating Scale-Revised (CDRS-R). Parents (P) and subjects (S) completed 10-item versions of the General Behavior Inventory Mania (GBI-M10) and Depression (GBI-D10) scales. Cohen's d was used to quantify effect sizes (positive values signify that the effect favored aripiprazole vs. placebo).

Results: For the YMRS total score, d = 0.79 pooled across the two aripiprazole treatment arms, corresponding to a large effect (d = 0.80 for 30 mg/day and 0.58 for 10 mg/day). Individual YMRS items' d -values ranged from 0.13 for thought content to 0.71 for disruptive/aggressive behavior. Seven items showed medium or large effects and four items showed small effects, including thought content, lack of insight (d = 0.26), bizarre appearance (d = 0.24), and hypersexuality (d = 0.18). The effect sizes for all parent-reported mania items were medium to large (0.41 for "depressed but high energy" to 0.78 for "rage combined with unusually happy") but were consistently small on subject self-reported items of mania and depression. Of note, all three of the depression scales (CDRS, PGBI-D10, SGBI-D10) contained individual items that demonstrated an effect in the opposite direction of the overall total score. For example, a small overall effect was seen on the CDRS-R (d = 0.12), but the items fatigue (d = -0.46) and hypoactivity (d = -0.28) demonstrated a notable effect in the opposite direction.

Discussion: In a trial of aripiprazole for the treatment of an acute manic or mixed episode associated with bipolar I disorder that showed a large effect on the primary outcome measure (the YMRS total score), there was marked heterogeneity in the effects detected on specific items on this scale and others used in the trial. Individual items shown to have poor psychometric properties in other published samples of the YMRS failed to detect treatment effects. Of note, across scales, irritability and aggression showed large treatment effects on both clinician and parent-reported measures, but less so for subject-reported measures. This perhaps reflects a lack of insight on the part of the subjects, which may be partly a reflection of the short-term nature of the trial. Items within all depression

measures showed contradictory effects, with the effect sizes of items assessing fatigue, energy, and increased sleep typically being in the opposite direction of items assessing symptoms of irritability. These contradictory effects reduced the overall effect size estimated by total scores. This pattern of findings is clinically plausible, but would not have been identified without analyzing change in individual items, as the depression items all show good internal consistency in baseline data. Results suggest that the YMRS total score in particular may be underestimating treatment effects in mania trials because of the psychometric properties of several of the items when used in pediatric samples. References: 1. *J Am Acad Child Adolesc Psychiatry*. 2002;41:1350-1359. 2. *J Am Acad Child Adolesc Psychiatry*. 2002;31:567-572.

Disclosure: E. Youngstrom: Part 1; Travel support from Bristol-Myers Squibb. J. Zhao: Part 5; Employee of Otsuka Pharmaceutical Development & Commercialization Inc. R. Mankoski: Employee of Bristol-Myers Squibb. C. Andersson: Employee of Bristol-Myers Squibb. R. McQuade: Employee of Otsuka Pharmaceutical Development & Commercialization Inc. R. Marcus: Employee of Bristol-Myers Squibb. R. Findling: Part 3; Received research support, acted as a consultant and/or served on a speaker's bureau for Abbott, Addrenex, AstraZeneca, Biovail, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, K.

192. Cariprazine, a D₃-preferring Dopamine D₃/D₂ Receptor Partial Agonist, Has Antidepressant-Like Activity With Fast Onset of Action in the Chronic Mild Stress-Induced Anhedonia Model

Nika Adham*, István Gyertyán, Béla Kiss, Gabriella Pasztor, Zsolt Szombathelyi, Marc G Caron, Piotr Gruca, Ewa Litwa, Mariusz Papp

Forest Research Institute, Jersey City, NJ

Background: Depression is widespread, chronic and recurring, associated with significant functional and social impairment, high levels of morbidity, and decreased quality of life. While antipsychotics are often used to treat bipolar mania, clinical evidence has also shown several of them to be effective in bipolar depression and in adjunctive treatment of depression. Cariprazine, a D₃-preferring dopamine D₃/D₂ receptor partial agonist, is a novel antipsychotic in Phase III clinical development for the treatment of schizophrenia and bipolar mania. Cariprazine has an approximate one order of magnitude higher selectivity for the dopamine D₃ receptor compared with the D₂ receptor. Since the D₃ receptor is thought to play a role in regulating mood, pharmacological treatment targeting this receptor may provide benefits in the treatment of depression. In the chronic mild stress (CMS) model, reduced intake of sucrose solution is observed in rats subjected to CMS indicating a behavior analogous to anhedonia, a hallmark characteristic of depression and a key negative symptom of schizophrenia. The ability of a compound to reverse reduced sucrose intake in the CMS model is thought to be highly predictive of antidepressant efficacy in humans. Earlier studies in this model demonstrated that the atypical antipsychotics olanzapine (2 mg/kg/day) and risperidone (0.5 mg/kg, BID), both D₂/5-HT_{2A} receptor antagonists, restored sucrose intake, with improvement reaching statistical significance by Week 5 for olanzapine ($P = <.01$) and Week 3 for risperidone ($P <.05$).¹ The aim of the present study was to evaluate if chronic treatment with cariprazine shows antidepressant-like effects in the CMS model; the tricyclic antidepressant imipramine and the atypical D₂ partial agonist antipsychotic aripiprazole were evaluated as reference compounds.

Methods: Male Wistar rats were exposed to a sequence of stressors (eg, food/water deprivation, cage tilting, stroboscopic illumination) for 2 weeks; nonstressed animals served as the control group. Stressed and nonstressed animals were then exposed to 5 consecutive weeks of continued stress or no stress (control group) in combination with drug or vehicle treatment. Intraperitoneal (IP) injections of vehicle, imipramine (10 mg/kg/day), aripiprazole (1 and 5 mg/kg/day) or

cariprazine (0.03, 0.065 and 0.25 mg/kg/day) were evaluated for their activity in reversing CMS-induced decrease in consumption of a 1% solution of sucrose. Statistical comparisons were made between stressed and control group animals, and between animals at Week 0 versus Weeks 1 to 5 of treatment.

Results: Consistent with previous results, CMS caused substantial reduction in the consumption of 1% sucrose solution. Imipramine fully reversed decreased sucrose intake, showing statistically significant improvement by Week 3 ($P = .05$). Aripiprazole was also effective in the CMS model ($P <.001$), significantly reversing stress-induced reduction of sucrose consumption at Week 1 (5 mg/kg, $P <.05$). All three doses of cariprazine tested (0.03, 0.065 and 0.25 mg/kg/day) significantly ($P <.001$) increased sucrose drinking in stressed animals, indicating antidepressant-like activity; no significant effects were observed in nonstressed (control) rats treated with cariprazine. The magnitude of effect for all doses of cariprazine in stressed rats was comparable to that of imipramine and aripiprazole. Cariprazine 0.065 mg/kg showed fast onset of action, reversing the deficit in sucrose consumption in stressed animals as early as Week 1 ($P <.01$).

Discussion: In the rat CMS model, both cariprazine and aripiprazole demonstrated faster onset of antidepressant-like action than the tricyclic antidepressant imipramine and the atypical antipsychotics risperidone and olanzapine. Cariprazine was more potent than aripiprazole in this model. These results suggest that cariprazine may be at least as effective as aripiprazole in depression. These behavioral data support further investigation of cariprazine for the treatment of depression. Clinical evaluation of antidepressant efficacy of cariprazine is ongoing. Additionally, the potential benefit of cariprazine in treating depression-like negative symptoms of schizophrenia (eg, anhedonia) are also being explored. Reference 1. Marston HM, Martin FD, Papp M, Gold L, Wong EH, Shahid M. Attenuation of chronic mild stress-induced 'anhedonia' by asenapine is not associated with a 'hedonic' profile in intracranial self-stimulation [published online ahead of print Aug 10, 2010]. *J Psychopharmacol*. doi: 10.1177/0269881110376684.

Disclosure: N. Adham: Part 5; Forest Research Institute. I. Gyertyán: Gedeon Richter Ltd. B. Kiss: Gedeon Richter Ltd. G. Pasztor: Gedeon Richter Ltd. Z. Szombathelyi: Gedeon Richter Ltd. M. Caron: Part 1; Acadia Pharmaceutical, Lundbeck A/G/USA, Forest Laboratories, Roche, NeuroSearch, GSK. Part 2; Acadia Pharmaceutical. Part 4; Lundbeck A/G/USA, Forest Laboratories, NeuroSearch. P. Gruca: None. E. Litwa: None. M. Papp: None.

193. A Novel Small Molecule Screening Approach to Discover Epigenetic Regulators as Therapeutics for Angelman Syndrome

John A. Allen*, Hsien-Sung Huang, Jayalakshmi Miriyala, Benjamin D. Philpot, Bryan L. Roth

Departments of Pharmacology and Molecular Physiology, and National Institute of Mental Health-Psychoactive Drug Screening Program, University of North Carolina, School of Medicine, Chapel Hill, NC

Background: Angelman syndrome is a neurodevelopmental disease and autism spectrum disorder that arises from allele specific loss of the gene UBE3A. Angelman syndrome is a classic example of a neurological disorder arising from genetic imprinting. UBE3A encodes an E3 ubiquitin ligase that is expressed in a parent-of-origin-specific manner; expression arises only from the maternal allele while the paternal allele is silenced through epigenetic mechanisms. Therefore, deletion or mutation of the maternal UBE3A gene is sufficient to eliminate Ube3a protein expression from most neurons during development and this results in Angelman syndrome. However, this neuronal imprinting phenomenon also suggests that unsilencing the intact, but quiescent, paternal UBE3A allele could be a potential therapy for Angelman syndrome. To this end, we have designed a novel small molecule screening approach to identify compounds that are capable of unsilencing the paternal UBE3A allele.

Methods: Primary cortical neurons from transgenic mice that encode a yellow fluorescent Ube3a fusion protein (Ube3a-YFP) were isolated and cultured in a 384 well plate format. A high-content-imaging-based screening assay was developed that detects fluorescence of Ube3a-YFP in individual neurons. An automated imaging and analysis system was also developed to assess maternal or paternal Ube3a-YFP levels before and after test compound treatments. As a proof-of-concept trial, numerous drugs that alter the epigenome were tested for activity (e.g. SAHA, valproic acid, 5-aza-2'-deoxycytidine, select HDAC inhibitors and DNA methyltransferase inhibitors).

Results: The high content screening platform successfully detected Ube3a-YFP fluorescence from >1200 individual neurons/well and reliably differentiated between active and silent UBE3A alleles in primary cortical neurons. The screening assay provided a robust assessment of Ube3a-YFP proteins levels in an automated fashion and exhibited an excellent Z' factor of 0.56. Unexpectedly, none of the epigenetic-related compounds tested to date have exhibited activity. We are now performing a comprehensive drug screen using multiple chemical libraries to identify small molecules that unsilence paternal UBE3A.

Discussion: The unique biology of UBE3A imprinting in Angelman syndrome suggests that pharmacological unsilencing of the paternal UBE3A gene would restore expression of Ube3a protein to the nervous system; this could be an effective therapeutic strategy to treat the disease. Since the molecular mechanisms, signaling pathways or enzymes controlling UBE3A imprinting are currently unknown, an unbiased, discovery-based screening approach is well-suited for identifying epigenetic regulators and molecules that unsilence UBE3A. We have now devised and validated a screening method that reports the allele-specific expression of UBE3A. This novel screening approach should facilitate the discovery of potential therapeutics for Angelman syndrome as well as identify compounds that are epigenetic regulators of genomic imprinting in the nervous system.

Disclosure: J. Allen: None. H. Huang: None. J. Miriyala: None. B. Philpot: None. B. Roth: None.

194. Effects of Adenosine A_{2A} Receptors Stimulation on Cocaine-Seeking Behavior

Ryan Bachtel*, Casey O'Neill, McKenzie Letendre

University of Colorado, Boulder CO

Background: Relapse to drug seeking is induced by exposure to drug-associated cues and pharmacological stimuli that activate the mesolimbic dopamine system. Chronic cocaine self-administration increases behavioral responses mediated by dopamine D₂ receptors and reinstatement of cocaine seeking is elicited by D₂ receptor stimulation. Temperance enhanced D₂ receptor-mediated behaviors may provide an effective treatment for curbing relapse susceptibility. Adenosine functions as a neuromodulator of dopamine neurotransmission and represents a target for inhibiting dopamine neurotransmission given the co-localization of adenosine A_{2A} and dopamine D₂ receptors on the same neurons within the nucleus accumbens. Previous work demonstrates that stimulation of adenosine A_{2A} receptors reduces both the development and expression of cocaine sensitization, impairs the initiation of cocaine self-administration and blunts cocaine seeking. The studies presented here test the hypothesis that stimulation of adenosine A_{2A} receptors localized to the nucleus accumbens will inhibit the reinstatement of cocaine seeking in extinguished animals.

Methods: Rats were trained to lever press for cocaine in daily self-administration sessions on a fixed-ratio 1 schedule for 3 weeks. After one week of abstinence, lever pressing was extinguished in 6 daily extinction sessions. We subsequently assessed the effects of localized nucleus accumbens core microinjections of the adenosine A_{2A} receptor agonist, CGS 21680, and the adenosine A_{2A} receptor

antagonist, MSX-3, in modulating cocaine- and quinpirole (D₂ agonist)-induced reinstatement to cocaine seeking.

Results: Intra-accumbens pretreatment of the A_{2A} agonist, CGS 21680, blocked both cocaine- and D₂-induced reinstatement, supporting our previous findings with systemic pretreatments of CGS 21680. Intra-accumbens treatment with the A_{2A} antagonist, MSX-3, on the other hand, showed a modest induction of cocaine seeking when given alone. An intra-accumbens pretreatment with MSX-3 exacerbated both cocaine- and D₂-induced reinstatement. Interestingly, the exacerbation of cocaine reinstatement produced by MSX-3 was only observed at subthreshold doses of cocaine and quinpirole suggesting an enabling of dopamine receptor stimulation through the removal of tonic adenosine stimulation.

Discussion: These findings demonstrate that stimulation of adenosine A_{2A} receptors blunts the ability of cocaine and dopamine D₂ receptor stimulation to reinstate cocaine seeking. In addition, removing the reciprocal regulation of D₂ receptors with an A_{2A} antagonist exacerbates cocaine seeking. Together, these findings suggest that adenosine A_{2A} receptor stimulation oppose the dopamine D₂ receptor signaling in the NAc that mediates cocaine relapse.

Disclosure: R. Bachtel: None. C. O'Neill: None. M. Letendre: None.

195. Cortical Glial Glutamate Transport (EAAT2) Contributes to the Response to Stress and to the Antidepressant Action of Ceftriaxone and Riluzole

Mounira Banasr*, Ashley Lepack, Ronald Duman, Gerard Sanacora

Yale University, New Haven, CT

Background: Growing evidence implicates glia dysfunction and abnormal glutamatergic neurotransmission in the neuropathology of stress-related illness including major depressive disorder. Postmortem studies have repeatedly found a decreased density and number of glia in cortical regions, including the prefrontal cortex (PFC) from depressed patients. We previously demonstrated that chronic stress resulted in a reduced glutamate/glutamine cycling and glial function in the PFC as well as depressive-like behavioral deficits. In addition, reduced expression and/or activity of the high affinity glial glutamate transporter GLT-1 (or EAAT2) were reported in rodent models of depression based on repeated stress. We hypothesized that two drugs, riluzole and ceftriaxone known to increase GLT-1 expression, have antidepressant-like effects in chronically stressed animals and that cortical GLT-1 increased activity is necessary for antidepressant action of these drugs.

Methods: Using rodent behavioral models that measure antidepressant efficacy, such as forced swim test and stress-induced anhedonia, we characterized the effects of riluzole and ceftriaxone in rats and mice in normal and stress conditions. Then, both genetic and pharmacologic tools were employed to address the specific role of GLT1 (EAAT2) transporter in the behavioral effect of stress and antidepressant properties of glutamate-modulating agents. Using heterozygous GLT1 knockout mice and GLT1 inhibitor infusions into the rat PFC, we were able to test the hypotheses of an increased sensitivity to stress in animals with altered GLT1 function and of requirement of GLT1 for riluzole and ceftriaxone antidepressant action. *In vitro*, we also tested whether similar changes can be found on primary cortical neuronal culture after corticosterone, riluzole and ceftriaxone exposure on mitochondrial metabolism and cell death and whether these neuroprotective effects involves GLT1.

Results: We first confirmed that riluzole and ceftriaxone chronic treatment increase GLT-1 protein levels in the rodent prefrontal cortex and demonstrated that both treatments reversed chronic stress-induced anhedonia. We also found that GLT1 heterozygote mice (GLT1-/+) show an exacerbated response to chronic stress in sucrose consumption. Indeed, 3 weeks of chronic unpredictable stress induced a significantly higher decrease (-45%) in daily sucrose consumption of GLT1 -/+ mice when compared to wildtype littermates. In addition we demonstrate that GLT1 -/+ mice show an attenuated response to

riluzole. In addition, local infusions of DHK (dihydrokainate, a selective GLT-1 inhibitor) in rat PFC induced an exacerbated response to stress and blocked the antidepressant-like effect of riluzole and attenuated the effect of ceftriaxone. *In vitro*, we also analyzed the consequence of the corticosterone on mitochondrial metabolism and cell death and confirmed that corticosterone exposure induced deleterious effects on these parameters. This effect was mediated by the glucocorticoid receptors since dexamethasone induced similar results. In addition, riluzole or ceftriaxone exposure prevented the effect of corticosterone administration. In parallel, we analyzed the consequences of reduced GLT-1 function on corticosterone-response; we found that dihydrokainate (DHK) administration exacerbates the corticosterone-induced reduction of mitochondrial metabolism and/or increased cell death. Our results suggest that we can model in culture some aspects of the stress/stress hormone action and that riluzole and ceftriaxone can block or attenuate these effects by enhancing of GLT-1.

Discussion: These data suggest that glia-mediated glutamate uptake in the PFC may contribute to the behavioral and physiological response to stress and demonstrate that riluzole's and ceftriaxone's antidepressant-like effects involve enhancement of cortical glutamate uptake. These studies support the hypothesis that glial dysfunction observed in the cortex tissue samples from patients with major depressive disorder may not only be a consequence of the illness but could also be a susceptibility factor for depression. Together, these results suggest modulation of glial mediated glutamate clearance may be a viable target for future antidepressant drug development.

Disclosure: M. Banasr: None. A. Lepack: None. R. Duman: None. G. Sanacora: Part 1; Abbott, AstraZeneca, Bristol-Myers. Part 2; Squibb, Evotec, Eli Lilly & Co., Roche, Novartis and Sepracor Inc.

196. HIV Infectivity of T Cells Is Increased by Depression and Decreased by a Functional Glucocorticoid Antagonist (RU-486)

Joshua Blume*, Tami D. Benton, Benoit Dube, David R. Gettes, Jian P. Lai, Kathleen Collins, Benjamin Cobb, Donald E. Campbell, Dana A. Brown, Tiffany Brown-Dominique, David Flanagan, Kevin Lynch, David Metzger, Steven D. Douglas, Dwight L. Evans

University of Pennsylvania, Philadelphia PA

Background: Glucocorticoids have been implicated in the pathophysiology of both depression and HIV/AIDS. Elevated serum cortisol occurs in HIV seropositive and depressed (seronegative) subjects in comparison to healthy controls. RU-486, a functional antagonist of the glucocorticoid receptor, has shown preliminary efficacy in the treatment of psychotic depression. Depression is a frequent comorbidity of HIV and is associated with accelerated HIV illness progression. Glucocorticoid dysfunction may partly mediate this association. We therefore investigated the possibility that a glucocorticoid antagonist (through direct action on cells of the peripheral immune system) may be a pharmacological strategy in the treatment of HIV. Using an *ex-vivo* model, we sought to determine whether the presence and degree of depressive symptoms in HIV seronegative subjects influenced viral replication following acute infection of their T-cells with HIV-1 (IIIB strain). We also determined whether treatment of subjects' T-cells with RU-486 attenuated HIV-1 infectivity and, if so, whether the effect of RU-486 varied according to the presence and severity of depression in the donor subjects.

Methods: 49 depressed and non-depressed HIV-negative women ($n=31$) and men ($n=18$), ages 18-57, underwent psychiatric assessment including a structured diagnostic interview and a 17 item Hamilton Depression Rating Scale (HDRS). Subjects were excluded if they had medical comorbidities, a psychotic disorder, current alcohol or substance disorders, or if they had taken psychotropic or immunomodulatory drugs in the eight weeks prior to assessment. For each subject, blood was drawn and monocyte-depleted peripheral blood mononuclear cells (PBMC's) were separated into two plates: one plate (treatment) was incubated with RU-486 and infected *ex-vivo*

with the (T-cell preferring) IIIB strain of the HIV virus; the other plate (control) was also infected with virus, but received no drug. At day six post-infection, viral loads of the treated and untreated cells were quantified.

Results: There was a direct association between increased HDRS score and viral load elevation in the untreated cells ($p<0.05$). When women and men were considered separately, the association remained significant in the women subgroup ($p<0.01$), but not in the men subgroup ($p=0.92$). Treatment with RU-486 reduced HIV viral load compared to no treatment ($p<0.001$). When women and men were considered separately, the effect was significant both in the women subgroup ($p<0.01$) and in the men subgroup ($p<0.02$). There was a direct association between the magnitude of the reduction in viral load and the HDRS score ($p<0.03$). This association was significant in the women subgroup ($p<0.001$), but not in the men subgroup ($p=0.73$).

Discussion: Depression is associated with increased susceptibility of T-cells to the HIV virus *ex-vivo*. HIV infectivity is attenuated by RU-486 *ex-vivo*, and the effect of RU-486 on viral load is greater as depression symptom burden increases. Further studies are warranted to elucidate the mechanisms mediating the effects of both depression and glucocorticoid antagonism on HIV infectivity, and to determine whether these effects differ in men and women.

Disclosure: J. Blume: None. T. Benton: None. B. Dube: Part 1; Boehringer Ingelheim Speakers Bureau. D. Gettes: None. J. Lai: None. K. Collins: None. B. Cobb: None. D. Campbell: None. D. Brown: None. T. Brown-Dominique: None. D. Flanagan: None. K. Lynch: None. D. Metzger: None. S. Douglas: None. D. Evans: PamLab.

197. Conditional Deletion of p38-Alpha MAPK in Serotonergic Neurons Blocks Stress-Induced Behavioral Responses.

Michael Bruchas*, Dan Messinger, Abbie Schlinder, Haripriya Shankar, Benjamin Land, Julia Lemos, Mayumi Miyatake, Catherine Hagan, John Neumaier, Charles Chavkin

University of Washington, Seattle, WA

Background: Our prior work has demonstrated that p38 mitogen-activated protein kinase (MAPK) activation by the dynorphin kappa opioid (KOR) system is a key mediator of the aversive responses caused by behavioral stress exposure. Numerous groups have also demonstrated that KOR antagonists are effective in blocking social defeat stress-induced immobility, swim-stress-induced immobility and stress-induced reinstatement to drug and alcohol seeking. Additionally, recent work has established a critical role for dorsal raphe, serotonergic accumbal KORs in stress-induced aversion and reinstatement of cocaine seeking, suggesting that the dynorphin KOR system mediates these behaviors through regulation of serotonergic tone. P38 MAPK is a highly conserved protein kinase and member of the tripartite MAPK family that has been shown to regulate serotonin transporter (SERT) function in prior *in vitro* biochemical studies, and stimulation of SERT by KOR-dependent activation of p38 MAPK is a plausible mechanism for stress-induced regulation of mood. However, whether p38 kinase regulates SERT function *in vivo* and subsequent behaviors is currently unknown.

Methods: In the present study, we used adeno-associated viral (AAV) and promoter-driven cre-recombinase expression to selectively excise floxed p38alpha MAPK in specific cell types and brain regions to understand the role of p38 activation in stress-induced behaviors.

Results: Injection of AAV1-cre (but not control vector containing inactive cre-recombinase) into dorsal raphe of floxed p38alpha MAPK mice, selectively disrupted p38alpha expression, blocked KOR-mediated conditioned place aversion (CPA), and prevented social defeat stress (SDS)-induced reinstatement of cocaine conditioned place preference (CPP). Using SERT promoter and Pet1-driven cre expression in floxed p38alpha MAPK to generate conditional knockout (CKO) mice, we also identified a fundamental role for serotonergic p38alpha in mediating these stress-induced behavioral responses.

Deletion of p38alpha MAPK in the serotonergic system via either SERTcre or PET1cre), blocked stress-induced CPA, produced an antidepressant-like effect in the forced swim test, and blocked stress-induced reinstatement to cocaine seeking. Social defeat stress also induced social avoidance, and we found that selective deletion of p38alpha in serotonin neurons blocked stress-induced social avoidance. Because the serotonin transporter (SERT) has been suggested to be a p38 MAPK substrate, we assessed SERT function using rotating disk electrode voltammetry (RDEV) in synaptosomes isolated from stressed and unstressed control mice. Pharmacological and stress-induced KOR activation increased 5HT transport through SERT by a p38alpha MAPK dependent process.

Discussion: Together these results suggest that activation of the p38alpha MAPK signaling cascade in the dorsal raphe and its projection fields is required for KOR-dependent stress-induced behaviors, including aversion and reinstatement. Supported by R21DA25970 and K99-DA25182.

Disclosure: M. Bruchas: Part 1; NIDA. D. Messinger: None. A. Schlinder: NIDA. H. Shankar: NIDA. B. Land: None. J. Lemos: NIMH. M. Miyatake: None. C. Hagan: None. J. Neumaier: NIDA, NIMH. C. Chavkin: NIDA.

198. The Antidepressant and Anxiolytic Properties of GLYX-13: A Novel NMDA Receptor Glycine Site Functional Partial Agonist

Jeffrey Burgdorf*, Ligia Westrich, Jeffrey Sprouse, Joseph Moskal

Falk Center for Molecular Therapeutics, Dept. of Biomedical Engineering, McCormick School of Engineering, Northwestern University & Naurex Inc., Evanston, IL

Background: Recent human clinical studies with known NMDA Receptor (NMDAR) antagonists CP-101,606 and ketamine have found significant reductions in depression scores in patients with treatment-resistant depression. Ketamine was also shown to produce a robust antidepressant effect in patients with treatment-resistant bipolar disorder. Although these drugs produced clinically unacceptable dissociative side effects, the efficacy in these studies was significant (>50% response rate in resistant subjects, fast onset of action, and long duration of effect up to 7 or more days following a single dose), and confirmed NMDAR as a novel target of high interest in depression. GLYX-13 is a tetrapeptide (TPPT) NMDAR functional glycine site partial agonist (GFPAs). It has been shown to enhance learning in young adult and learning-impaired aging rats. GLYX-13 is unique among NMDAR modulators in its ability to simultaneously elevate long-term potentiation (LTP) while reducing long-term depression (LTD). In this report, GLYX-13 was examined for its potential as a clinically relevant antidepressant using the rat Porsolt test, open field test, and hedonic ultrasonic vocalization (USVs) test. We also tested for ketamine-like addictive, dissociative, and sedative side effects in the rat place preference, pre-pulse inhibition (PPI), and open field tests. The dependence of AMPA receptor activation for the prolonged antidepressant effect of GLYX-13 was tested by: (1) the ability of GLYX-13 to upregulate AMPA GluR1, glycogen synthase kinase 3 beta (GSK-3 β), and alter levels of beta-catenin (a substrate for GSK-3 β) in the medial prefrontal cortex; and (2) the ability of the AMPAR antagonist NBQX to block the antidepressant effects of GLYX-13.

Methods: *Behavioral Pharmacology:* Male Sprague-Dawley (SD) rats (2-3 Months old) were given injections of GLYX-13 (i.v., intranasal, intra-MPFC), ketamine (10 mg/kg i.p. or i.v.) or sterile 0.9% saline vehicle 20-60 min before the start of testing. Antidepressant-like drug effects were measured by decrease in floating time in the Porsolt test. Anxiolytic-like drug effects were measured by increased center time in the open field. Sedation behavior was measured by open field line crosses. Hedonic drug effects were measured by increased rates of 50-kHz hedonic USVs during heterospecific play. Prepulse inhibition and place preference studies were also conducted. *Molecular Pharmacology:* Male SD rats were given i.v. injections of GLYX-13

(3 mg/kg) or ketamine (10 mg / kg) and sacrificed 24 hrs post injection without behavioral testing. Total and phospho GLUR1, total and phospho GSK-3B and total beta-catenin protein levels were measured in the medial prefrontal cortex by western blot or ELISA. The ability of NBQX to inhibit the antidepressant effect of GLYX-13 in the Porsolt test was also examined. *Phase I Clinical trial:* GLYX-13 was administered to normal human volunteers as a single dose (0.5, 1, 5, 10 mg / kg i.v.). Potential dissociative symptoms were measured and plasma bioavailability of GLYX-13 was calculated.

Results: *Behavioral Pharmacology:* GLYX-13 (1) produced an anti-depressant-like effect in the Porsolt test 20 min to 2 weeks post injection, (2) produced an anxiolytic-like effect in the open field test, (3) increased rates of hedonic 50-kHz USVs, and decreased rates of aversive 20-kHz USVs, and (4) GLYX-13 did not show any ketamine-like abuse potential in the place preference test, schizophrenia-like effects in the PPI, or sedative effects in the open field. *Molecular Pharmacology:* GLYX-13 and ketamine increased MPFC phospho-GLUR1 and -GSK-3B levels and decreased beta-catenin levels. The antidepressant-like effects of GLYX-13 were blocked by the AMPA receptor antagonist NBQX. *Phase I Clinical trial:* In normal human volunteers, GLYX-13 did not produce any ketamine-like dissociative side effects, and GLYX-13 was found to have equal or greater exposure (Cmax and AUC) in humans as in rats.

Discussion: The data reported here show that GLYX-13 displays significant antidepressant-like and anxiolytic-like properties in rats by an AMPA dependent mechanism similar to ketamine and electro-convulsive shock therapy (ECT), but without the dissociative side effects. In contrast to SSRIs, GLYX-13's onset of action was within minutes of a single dose and treated both the deficits in positive affect and negative affect associated with depression. Thus, GLYX-13 is an attractive candidate for the treatment of depression. GLYX-13 has an open IND, is being developed by Naurex, Inc., and is now entering Phase II clinical trials for the treatment of depression.

Disclosure: J. Burgdorf: Part 1; Naurex Inc. L. Westrich: Part 5; Lundbeck Research USA. J. Sprouse: Lundbeck Research USA. J. Moskal: Part 3; Naurex Inc.

199. Effects of the Unbalanced Triple Transporter Inhibitor EB-1010 on Monoamine Uptake Transporters *in Vitro* and *in Vivo*

Frank Bymaster*, Anthony McKinney

Euthymics Biosciences Inc, Cambridge, MA

Background: The current pharmacotherapy of major depressive disorder is less than ideal with remission rates of 30-40%. In addition some symptoms such as cognitive impairment, anhedonia, fatigue and sleepiness are not markedly improved and, many of the drugs have troublesome adverse events such as sexual dysfunction, weight gain, and possibly cognitive impairment. Enhancement of dopamine (DA) neurotransmission such as with bupropion has been shown to positively impact these poorly-treated symptom domains as well as mitigate sexual dysfunction and weight gain. Thus, a compound incorporating inhibition of serotonin (5-HT), norepinephrine (NE), and DA transporters could be a marked improvement in the pharmacotherapy of MDD. Such a compound is EB-1010 (formerly DOV-21,947, [(+)-1-(3,4-dichlorophenyl)-3-azabicyclo-[3.1.0]hexane hydrochloride] which has been shown to an unbalanced triple uptake transporter inhibitor (TUI) with ratios of 1:2:8 for inhibition of 5-HT, NE, and DA uptake transporters (Skolnick et al, 2003). It was orally active in the rat forced swim and mouse tail suspension models of depression with a minimal effective dose of 5 mg/kg. The compound also reduced body weight in diet-induced obese rats without significantly affecting major organ systems such as the cardiovascular system (Tizzano et al., 2007). After oral administration to rats, EB-1010 had linear and high plasma exposure, a plasma elimination half-life of 2.6 h, a T_{max} of 1-2 hours, 99% plasma protein binding, and 77% bioavailability. Here we further characterize the pharmacology of EB-1010 in *ex vivo* binding assays, microdialysis studies evaluating effects

on extracellular levels of monoamines, locomotor activity, cytochrome P450 isoenzymes, and hERG channel interactions.

Methods: Inhibition of *ex vivo* binding in rats was determined by Lengyel et al., 2008. Effects of EB-1010 on concentrations of monoamines and their metabolites was determined using standard microdialysis studies in rat brain regions, and locomotor and stereotypic activity was evaluated in activity cages. Interaction with cytochrome P450 isoenzymes and the hERG ion channel was determined using standard assays.

Results: EB-1010 inhibited *ex vivo* binding to 5-HT, NE and DA transporters in a dose-related fashion and consistent with its *in vitro* binding profile. EB-1010 at a behaviorally active dose (10 mg/kg ip.) markedly increased extracellular concentrations of 5-HT, NE and DA in rat prefrontal cortex and DA in striatum in a time-dependent fashion, thus demonstrating TUI activity *in vivo*. The dialysate concentrations of the metabolites of 5-HT and DA, 5-hydroxyindole-acetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC), respectively, were significantly reduced by EB-1010, consistent with uptake blockade without release. Locomotor studies demonstrated that EB-1010 did not stimulate motor activity or stereotypic movements from doses of 5-40 mg/kg po, or 8 times the behaviorally active dose. The potential of EB-1010 to have drug-drug interactions due to cytochrome P450 isoenzyme inhibition was evaluated and the drug had low affinity for the key isoenzymes CYP2D6 and CYP3A4. The only isoenzyme with marked EB-1010 affinity was CYP2B6 ($K_i = 1.8 \mu\text{M}$). In electrophysiological studies, EB-1010 had concentration-dependent inhibition of the hERG (human ether-a-go-go gene) channel (I_{kr}) current with an IC_{50} of $4.6 \mu\text{M}$ and a calculated safety margin of 74.

Discussion: EB-1010 inhibited *ex vivo* binding to 5-HT, NE and DA receptors and the effects were consistent with its *in vitro* binding profile. EB-1010 also increased extracellular concentrations of the three monoamines in brain regions, consistent with TUI activity. Although the compound increased extracellular concentrations of DA, it did not stimulate locomotor activity or stereotypies, suggesting low likelihood to have drug abuse potential. The 1-2 h T_{max} as well as 5-HT and NE interactions likely contributed to the lack of locomotor stimulation. EB-1010 did not cause marked inhibition of major CYP450 isoenzymes and had an adequate safety margin at the hERG ion channel. Overall, these data indicate that EB-1010 is an unbalanced TUI *in vitro* and *in vivo* with good druggability and safety properties. EB-1010 is an appropriate drug candidate for MDD to evaluate the effects of modest enhancement of DA in addition to facilitating 5-HT and NE neurotransmission. References: Lengyel et al., *Neuropharmacology*. 2008, 55(1):63-70. Skolnick et al., *Eur J Pharmacol*. 2003, 461:99-104. Tizzano et al., *J Pharmacol Exp Ther*. 2008, 32:1111-26.

Disclosure: F. Bymaster: Part 5; Euthymics Biosciences Inc. A. McKinney: Euthymics Biosciences Inc.

200. Cocaine-Seeking Behavior Blocked for 6 Months in Rats Treated with Cocaine Hydrolase Delivered by a Viral Vector

Marilyn Carroll*, Stephen Brimijoin, Yang Gao, Robin Parks, Natalie Zlebnik, Paul Regier, Justin Anker

University of Minnesota, Minneapolis, MN

Background: Cocaine dependence is a devastating and chronic disorder characterized by high rates of relapse. In our previous study, direct administration of a quadruple mutant albumin-fused butyrylcholinesterase (BChE)-based enzyme, termed albumin-cocaine hydrolase (Albu-CocH) acutely blocked cocaine seeking in an animal model of relapse and abolished cocaine-induced seizures and lethality. In the present experiment we extended these results to a gene transfer approach using a related BChE mutant termed "AME". Our goal was to determine if a single iv delivery of an adenoviral vector encoding this enzyme would block the reinstatement of cocaine seeking for an extended period of time.

Methods: Male and female rats were trained to self-administer 0.4 mg/kg cocaine under a fixed-ratio 1 schedule of reinforcement, and they

maintained this behavior for approximately 10 days. Following the final self-administration session, rats were injected iv with saline or AME vector, and their cocaine solutions were replaced with saline. Rats were then allowed to extinguish lever pressing for 14 days while only saline was available. Subsequently, they were tested for drug-primed reinstatement during an 8-day procedure in which ip priming injections of saline (S), cocaine (5, 10, and 15 mg/kg, C), and amphetamine (A) were administered according to the following sequence: S, C, S, C, S, C, S, A. Cocaine priming injections were then administered once weekly for 4 weeks and then once monthly for up to 6 months following the single AME vector or vehicle treatment to assess long-term effects on cocaine seeking, and an amphetamine priming injection was also given at 6 months. Plasma enzyme levels were analyzed at various time points.

Results: Prior to the AME vector or saline treatment, groups did not differ in the number of cocaine infusions self-administered during maintenance or in the number of saline infusions self-administered during extinction. However, females showed more cocaine self-administration than males on some of the maintenance days and more saline infusions on the first day of extinction indicating more resistance to extinction. Cocaine-primed reinstatement responding was significantly suppressed by the AME vector for up to 6 months following treatment compared to saline-treated controls. In contrast, rats in both the AME vector and saline control groups showed marked reinstatement to an amphetamine priming injection when tested 3 weeks after cocaine self-administration and again after 6 months, indicating that the AME vector specifically blocked cocaine-primed reinstatement, and behavior was not generally suppressed by enzyme treatment. Also, analysis of plasma samples indicated that the AME vector produced substantial and sustained cocaine hydrolase activity.

Discussion: This is the first demonstration of an effective, long-term treatment for cocaine abuse that was sustained by a viral vector for at least 6 months after a single injection. No adverse side effects were noted that distinguished the enzyme-treated vs control groups. Rats showed robust reinstatement responding after a priming injection of amphetamine, indicating no general decrement in behavior due to treatment. Initial results also suggest a sex difference in treatment effectiveness, with females showing a greater reduction in cocaine-seeking than males. These results suggested that viral transfer of cocaine hydrolase may be useful as a long-term treatment for preventing relapse to cocaine addiction in humans. This work was supported by R01 DA023979-03, R01 DA023979-03S1 (SB and MEC), and K05 DA15267-08 (MEC).

Disclosure: M. Carroll: None. S. Brimijoin: None. Y. Gao: None. R. Parks: None. N. Zlebnik: None. P. Regier: None. J. Anker: None.

201. Role of cJun C-Terminal Kinase (JNK) Isoforms in Ligand-Directed Mu and Kappa Opioid Receptor Inactivation

Charles Chavkin*, Erica Melief, Mayumi Miyatake, Michael Bruchas, Selena Schreiber, Haripriya Shankar

University of Washington, Seattle, WA

Background: Prolonged exposure to uncontrollable stressors increases the risk of mood disorders and drug addiction by mechanisms that may involve stress-induced activation of the dynorphin / kappa opioid system. Pharmacological activation of the kappa opioid receptor evokes dysphoria in humans and aversion behaviors in rodents. Mice stressed by either repeated forced swim or repeated social defeat showed a strongly potentiated response to the rewarding properties of cocaine in the conditioned place preference (CPP) assay. The potentiation was blocked by the kappa antagonist norBNI or by deletion of the kappa receptor or dynorphin genes. Similarly stress-induced reinstatement of extinguished cocaine CPP depended on activation of the dynorphin/kappa opioid system. Although preclinical studies suggest their therapeutic utility in treating stress-disorders, selective KOR antagonists generally have very long-durations of effect. The goal of this study was to elucidate the mechanisms responsible for these long-lasting effects.

Methods: The duration of antagonist effect in male C56Bl/6 mice was assessed after administration of drug followed by subsequent *in vivo* challenge with opioid analgesic prior to measuring mu or kappa opioid induced anti-nociception (increase in tail flick latencies). Direct effects of opioids on G protein coupling to MOR and KOR was assessed by *in vivo* treatment followed by subsequent *ex vivo* stimulation by DAMGO or U69,593 of [³⁵S]-GTPyS binding to membranes isolated from mouse spinal cord.

Results: Two classes of kappa opioid antagonists can be distinguished: short acting antagonists (including naloxone, naltrexone and buprenorphine) produced acute antagonism of the analgesic effects of the selective kappa antagonist U50,488 that completely reverse by 24 hrs. Long acting kappa antagonists (norBNI, GNTI and JDtic) produced both acute antagonism and inhibitory effects that persisted for more than 2 weeks. The long-lasting antagonism could be blocked by inhibitors of c-Jun Kinase (JNK). New data presented here showed that the long lasting effects were not evident in JNK1 knockout mice and could also be blocked by PKC inhibitor Gö6976. In contrast, ERK inhibitors and JNK2 knockout were ineffective. New data from GTPyS binding studies showed that the inhibitory effect of JNK activation was at the level of the receptor: long acting antagonists reduced kappa opioid stimulation of GTPyS binding to spinal cord membranes by U69,593 7-days after *in vivo* treatment without affecting kappa receptor number or binding affinity. The inhibition of GTPyS binding was blocked by treatment with the JNK inhibitor SP600125 prior to norBNI. Previous studies suggested a role for PKC in tolerance to morphine, but not to other MOR agonists. To determine the relationship between PKC and JNK-mediated effects, we used the small molecule PKC inhibitor Gö6976 in HEK293 cells expressing rMOR-GFP or rKOR-GFP prior to treatment with morphine or norBNI, respectively. The increased phospho-JNK-ir caused by both morphine and norBNI was blocked by pretreatment with Gö6976. Together, these results indicate that the JNK1 isoform mediates ligand-directed inactivation of the KOR, whereas the JNK2 isoform mediates inactivation of MOR and activation of JNK may require PKC.

Discussion: These results support the concept that ligand-directed signaling is evident at both MOR and KOR. Kappa opioid receptor activation (by norBNI-like antagonists) or Mu opioid receptor activation (by morphine-like agonists) initiate a PKC-JNK signaling cascade that results in inhibition of G-protein activation. For MOR this manifests as acute analgesic tolerance to morphine, and for KOR this manifests as non-competitive antagonism. The long-lasting inhibition by norBNI-like antagonists suggests that the changes in KOR signal complex are stable. Persistent antagonism might result from the association of a JNK-substrate that sterically blocks G-protein access, but the presence of a hypothetical jamming protein has not yet been demonstrated. The therapeutic potential of kappa opioid antagonists has been suggested by a series of preclinical studies using assays of stress-induced anxiety and depression-like behaviors. However, rather than being standard anti-anxiety or antidepressant drugs, we predict that kappa antagonists efficacy will be restricted to circumstances in which the endogenous dynorphin tone has been elevated, typically by stress exposure. KOR antagonists are more likely to promote stress-resilience than to act broadly as anxiolytics or general antidepressants. Supported by DA11672.

Disclosure: C. Chavkin: Part 1; Consultant, Adolor, Lilly, Sepracor. E. Melief: None. M. Miyatake: None. M. Bruchas: None. S. Schreiber: None. H. Shankar: None.

202. Sub-Anesthetic Ketamine Acutely Increases Glutamate-GABA-Glutamine Metabolism in Medial Prefrontal Cortex of Awake Rats

Golam Chowdhury*, Gerard Sanacora, Douglas, L Rothman, William Cho, Kevin Behar.

Yale University, New Haven, CT

Background: Ketamine, a non-competitive antagonist of the NMDA receptor, produces anesthesia with decreased metabolism at high doses

but at low doses is associated with cognitive deficits, perceptual alterations, delayed antidepressant-like effects and increased metabolism. Increasing evidence suggests the cognitive, perceptual and the antidepressant effects of low dose ketamine involve alterations of GABAergic and glutamatergic neurotransmission in prefrontal cortex (PFC). 2-Deoxyglucose studies have shown focal increases in glucose consumption in medial PFC (mPFC), although this method cannot differentiate between oxidative and non-oxidative metabolism. The effects of ketamine on neuronal oxidation or neuron-glia glutamate-GABA/glutamine cycling has not been reported. The objective of this study was to determine if an acute sub anesthetic dose of ketamine alters glutamate and GABA neurotransmitter cycling in a manner consistent with its proposed antidepressant-like effects.

Methods: Male Sprague-Dawley rats (~180-200 g) were prepared with tail vein catheters under isoflurane anesthesia for infusion of either [¹⁻¹³C]glucose or [²⁻¹³C]acetate. [¹⁻¹³C]glucose is metabolized mainly in the neuronal TCA cycle and labels neuronal glutamate and GABA, which are released and taken up by astrocytes, followed by conversion (and labeling) of glutamine. [²⁻¹³C]acetate is metabolized by astrocytes labeling glutamine, which is released and taken up by neurons for synthesis of glutamate and GABA. Rats were allowed to recover from anesthesia for at least 30 min prior to study. Rats received intraperitoneal injections of ketamine-HCl (30 mg/kg in 0.9% saline) or saline. Ten minutes after injection of ketamine or saline, rats received infusions of either [¹⁻¹³C]glucose for 8 min (n=6,5) or [²⁻¹³C]acetate for 15 min (n=5,5). The infusions yielded rapid and constant elevations of ¹³C labeled glucose or acetate concentrations and ¹³C enrichments in the blood. The short period of label infusion ensured that ¹³C incorporation into brain amino acid pools is proportional to the TCA cycle rate of the respective cell types. At the appropriate time rats were quickly sedated and euthanized by focused-beam microwave irradiation, stopping all further metabolism. The mPFC was carefully dissected and frozen in liquid nitrogen, along with heart blood drawn immediately after death. The brain tissue was extracted in methanol/HCl and ethanol, centrifuged, and the supernatants lyophilized. Samples were dissolved in phosphate buffered deuterium oxide. The concentration and ¹³C enrichments of glutamate, GABA and glutamine were determined using ^{1H-[¹³C]} NMR spectroscopy at 11.74 Tesla. Percentage ¹³C enrichments of brain amino acids were normalized by their respective blood ¹³C enrichment.

Results: Ketamine (30 mg/kg, i.p.) produced stereotyped progressive behavioral responses (back and forth head movements, ataxia, followed by hyperactivity) within minutes of injection consistent with previous reports. Total concentrations of glutamate (Glu), GABA, and glutamine (Gln) in mPFC were unaffected by ketamine (P>0.2). Preliminary analysis revealed that the percentage ¹³C enrichments of Glu-C4, GABA-C2 and Gln-C4 were higher in ketamine-treated rats infused with [¹⁻¹³C] glucose (15%, 25%, and 43%; p<0.02) or [²⁻¹³C]acetate (32%, 58%, and 15%; p<0.02), indicating that both neuronal TCA cycle rate and Glu/GABA-Gln cycling were increased by ketamine.

Discussion: Ketamine at sub-anesthetic doses acutely increases glutamate and GABA labeling from both [¹⁻¹³C]glucose and [²⁻¹³C]acetate in the rat medial prefrontal cortex, consistent with increased neuronal oxidative metabolism and increased neurotransmitter glutamate/GABA-glutamine cycling. Future studies will need to determine if the increased metabolism and amino acid neurotransmitter cycling in the PFC are associated with the perceptual, cognitive and behavioral effects of low dose ketamine administration.

Disclosure: G. Chowdhury: None. G. Sanacora: Part 1; Abbott, AstraZeneca, Bristol-Myers Squibb, Evotec, Eli Lilly & Co., Johnson & Johnson, Roche, Novartis and Sepracor Inc.. Part 2; Eli Lilly and Co.. Part 4; AstraZeneca, Bristol-Myers Squibb, Merck & Co., Roche, and Sepracor Inc.. D. Rothman: None. W. Cho: Part 5; Merck & Co., Inc.. K. Behar: None.

203. The MCH System as a New Target for Psychiatry

Shinjae Chung*, Michel Verheij, Mark Geyer, Gerard Martens, Olivier Civelli

UCIrvine, Irvine, CA

Background: Deficits in sensorimotor gating measured by prepulse inhibition (PPI) of startle have been known as characteristics of patients with schizophrenia and related neuropsychiatric disorders. Previous studies have found that the dopamine system plays an important role in PPI disruption in rodents as well as in human. Melanin-Concentrating Hormone (MCH) is mainly produced in the lateral hypothalamus and its receptors are highly expressed along the mesocorticolimbic dopamine pathway, in particular, the shell of the nucleus accumbens (NAcSh). MCH_{1R} is present in the circuitry in which dopamine system overactivity is thought to lead to schizophrenia. We examined whether the MCH system activity modulates the prepulse inhibition (PPI) of the startle reflex, directly or when disrupted by dopamine-related drugs, which serves as an animal model that is relevant to schizophrenia symptoms.

Methods: Startle reactivity was measured using startle chambers. PPI session consisted of startle, prepulse and no-stimulus trials and these were presented in a pseudorandom order. The amount of PPI was calculated as a percentage score for each acoustic prepulse trial type: % PPI = 100 - {[(startle response for prepulse + pulse)/(startle response for pulse-alone)]x100}.

Results: Regarding the prepulse inhibition (PPI) of startle, we found that there is no significant effect of central MCH injection on either startle or PPI level, although there was significant main effect of prepulse intensity on PPI. Because the mixed D₁/D₂ agonist apomorphine is known to disrupt PPI, we then tested whether MCH could affect apomorphine-induced PPI disruption and found that MCH dose dependently increased PPI deficit upon low doses of apomorphine. In contrast, central MCH injection did not affect stereotyped behaviors. We then used apomorphine-susceptible (APO-SUS) and apomorphine-unsusceptible (APO-UNSUS) rats to test whether modulating the MCH system activity in these rats affects PPI and stereotyped behaviors.

Discussion: We found that the MCH system can modulate dopamine-related responses. In sensorimotor gating, MCH is able to increases the disruptions induced by low doses of apomorphine. Because the MCH_{1R} is expressed at a very low level in the striatum, these data position the MCH system as a unique target for therapies directed at modulating the dopamine tone selectively in the nucleus accumbens.

Disclosure: S. Chung: None. M. Verheij: None. M. Geyer: None. G. Martens: None. O. Civelli: None.

204. Modulation of Neuropeptide S System Activity Regulates Cue Induced Reinstatement of Cocaine Seeking: An Effect Mediated by Hypothalamic Hypocretin-1/Orexin-A System

Roberto Ciccocioppo*, Marsida Kallupi, Giordano De Guglielmo, Barbara Ruggeri, Nazzareno Cannella, Laura Soverchia, Massimo Ubaldi, Serena Stopponi, Markus Heilig

Camerino Univ, Camerino, Italy

Background: Cocaine addiction is a chronic relapsing disorder and environmental conditioning factors are one of the major determinants of relapse in abstinent cocaine users.

Methods: Here we describe the role of Neuropeptide S (NPS) system modulation in regulation of cue-induced cocaine seeking.

Results: In rats with a history of cocaine self-administration, presentation of stimuli predictive of drug availability reinstates drug-seeking. Intracerebroventricular (ICV) injection of Neuropeptide S (NPS) increased this conditioned relapse-like behavior, while peripheral administration of the NPS receptor antagonist SHA 68 reduced

it. Follow up studies showed that site specific brain microinjection of NPS reduced cue-induced cocaine seeking after administration into the lateral hypothalamus (LH) and the perifornical area (PeF) but not into central amygdala (CeA). Consistent with this finding, administration of the selective NPS receptor antagonist [D-Cys(tBu)(5)]NPS or [(tBu-d-Gly(5)]NPS reduced cocaine seeking following injections directed to the LH and the PeF but not to the CeA. We also found that ICV NPS administration activates c-Fos expression in Hypocretin-1/Orexin-A (Hcrt-1/Ox-A) immunoreactive neurons in the LH and in the PeF. Of note, IP treatment with the selective Hcrt-1/Ox-A receptor antagonist SB334867 prevented the increase of cocaine seeking elicited by intra LH injection of NPS.

Discussion: Together, these findings indicate a role for the NPS system in the physiopathology of relapse to drug seeking, and suggest that NPS receptor antagonism may represent a new strategy for prevention of relapse to cocaine use. (Support: Compagnia San Paolo Foundation; NPS grant and intramural research funds of the NIAAA.)

Disclosure: R. Ciccocioppo: None. M. Kallupi: None. G. de Guglielmo: None. B. Ruggeri: None. N. Cannella: None. L. Soverchia: None. M. Ubaldi: None. S. Stopponi: None. M. Heilig: None.

205. The Effects of Chronic Paroxetine and Fluoxetine Exposure in Adolescent Rats

Cynthia Crawford*, Leslie Horn, Venuz Greenfield

California State University, San Bernardino, San Bernardino, CA

Background: Major depression is a common problem in adolescents. Unfortunately, many of the medications that are effective in adults at relieving the symptoms of depression are ineffective in adolescent populations. Moreover, the most popular class of antidepressants, the selective serotonin inhibitors (SSRIs), can induce suicidal thoughts in adolescents. The mechanism for this paradoxical increase in suicidal ideation and behavior is unknown and limited research has been conducted on this topic. Therefore, in the present investigation we assessed neurochemical measures associated with depression and suicidal behavior in adolescent male and female rats after chronic treatment with the SSRIs, paroxetine and fluoxetine.

Methods: Male and female Sprague-Dawley rats were injected with paroxetine (2.5 or 10 mg/kg), fluoxetine (10 mg/kg) or saline starting on postnatal day (PD) 35 for 10 consecutive days. All injections were given intraperitoneal at a volume of 1 ml/kg. The day after the last injection, rats were killed and the prefrontal cortex and hippocampus were removed. Norepinephrine and serotonin levels were then measured using high performance liquid chromatography with electrochemical detection and brain derived neurotrophic factor (BDNF) was measured using enzyme-linked immunosorbent assays (ELISA).

Results: In the prefrontal cortex, vehicle-treated female rats had greater levels of norepinephrine than male rats. There were no sex differences in serotonin levels. Both chronic paroxetine (10 mg/kg) and fluoxetine treatment (10 mg/kg) decreased serotonin levels in the prefrontal cortex. In contrast, chronic fluoxetine exposure increased norepinephrine levels but only in male rats. In the hippocampus, there were no sex differences in monoamine levels among vehicle treated rats. Chronic fluoxetine treatment did not alter levels of either serotonin or norepinephrine. Chronic paroxetine exposure (10 mg/kg) decreased serotonin levels in both male and female adolescent rats but not did not alter norepinephrine levels. BDNF levels were also reduced by chronic paroxetine treatment (10 mg/kg) in the hippocampus of both male and female rats. Chronic fluoxetine treatment did not alter BDNF levels of adolescent rats.

Discussion: The present data demonstrated that chronic treatment with the SSRIs, paroxetine and fluoxetine, decreased monoamine levels in the prefrontal cortex and hippocampus. Moreover, chronic treatment with paroxetine decreased levels of hippocampal BDNF.

These data suggest that SSRIs may potentiate depression in adolescents and provide a possible cause for the increase in suicidal ideation seen with SSRI treatment.

Disclosure: C. Crawford: None. L. Horn: None. V. Greenfield: None.

206. Selective mGluR5 Antagonism Attenuates the Stress-Induced Reduction of MK-801's Antiseizure Potency in the Genetically-Inbred Balb/c Mouse

Jessica A. Burket, Stephen Deutsch*, William R. Cannon, Luis Jacome
Eastern Virginia Medical School, Norfolk, VA

Background: The ability of MK-801 (dizocilpine), a noncompetitive NMDA receptor antagonist, to antagonize electrically-precipitated seizures (i.e., tonic hindlimb extension) is reduced 24 hours after mice are exposed to a single session of forced swimming in cold water. The current experiment explored the effect of MPEP (2-methyl-6-(phenylethynyl)-pyridine), an antagonist of the mGluR5 subtype of group 1 metabotropic glutamate receptors, administered intraperitoneally 10 minutes prior to exposure to stress (30 mg/kg) on the stress-induced reduction of MK-801's anti-seizure effect in two strains of mice: outbred Swiss-Webster and genetically-inbred Balb/c mice. The Balb/c mouse strain is hypersensitive to behavioral effects of MK-801 and shows deficits of sociability, compared to the outbred strain. The mGluR5 subtype of group 1 metabotropic glutamate receptors, a G_q-protein linked receptor that is positively coupled to phospholipase C, is functionally associated with the NMDA receptor, influencing the latter's phosphorylation state and channel properties. Moreover, the mGluR5 receptor may contribute to regulation of protein synthesis in the region of the basilar dendrite, which could explain the beneficial effects of MPEP in the *Fmr1* knockout mouse (KO), a transgenic mouse model of the Fragile X syndrome that is deficient in expression of the 'fragile x mental retardation protein (FMRP).' FMRP complexes with mRNA associated with basilar dendritic polyribosomes and, thereby, inhibits its translation; thus, mGluR5 stimulated protein synthesis is unopposed in the *Fmr1* KO mouse.

Methods: Experimentally-naive, 8-week old male outbred Swiss-Webster and genetically-inbred Balb/c mice were forced to swim in cold (6°C) water for up to 10 min 24 h prior to testing the ability of MK-801 to antagonize electrically precipitated tonic hindlimb extension. Groups of mice were treated with MPEP (30 mg/kg, ip) 10 minutes prior to the single session of forced swimming in cold (6°C) water. With the exception of one condition that contained 13 mice, each experimental condition was tested in groups of 12 mice.

Results: Post-hoc comparisons revealed that treating Balb/c mice with MPEP (30 mg/kg, ip) 10 minutes prior to a single session of forced swimming in cold (6°C) water attenuated the severity of the reduction of the anti-seizure effect of 1.0 mg/kg of MK-801 24 hours later ($p < 0.05$). This finding indicates that mGluR5 receptor antagonism prior to stress can modulate the severity of the stress-induced reduction of MK-801's anti-seizure potency.

Discussion: The mechanism through which treatment with MPEP prior to exposure to stress attenuates the reduction of MK-801's anti-seizure effect 24 hours later is not known. However, MPEP may alter the phosphorylation status of the NMDA receptor itself and, thereby, change its functional properties, making it more resistant to this effect of exposure to stress. Alternatively, MPEP may inhibit translation of a specific program of proteins in the local area of the basilar dendrite in response to stress that contributes to the reduced ability of MK-801 to antagonize electrically-precipitated seizures 24 hours after exposure. The data suggest that the mGluR5 receptor may serve as a therapeutic target for stress-related and other psychiatric disorders.

Disclosure: J. Burket: None. S. Deutsch: None. W. Cannon: None. L. Jacome: None.

207. Opposite Effects of Metabotropic Glutamate 5 and N-methyl-D-aspartate Receptor Antagonists Administered in the Nucleus Accumbens Shell on Nicotine Self-Administration in Rats

Manoranjan D'Souza*, Athina Markou

University of California San Diego, La Jolla, CA

Background: Tobacco smoking, a preventable cause of worldwide morbidity and mortality, is partly attributed to the reinforcing properties of nicotine contained in tobacco. The excitatory neurotransmitter glutamate plays an important role in the reinforcing effects of nicotine. Nicotine increases glutamatergic transmission by acting on excitatory nicotinic receptors located on presynaptic glutamatergic terminals. The postsynaptic actions of glutamate in turn are mediated by ionotropic (fast acting) and metabotropic (slow acting) receptors. Consequently, blockade of glutamatergic neurotransmission via systemic administration of the ionotropic N-methyl-D-aspartate (NMDA) receptor antagonist LY235959 or the metabotropic glutamate 5 (mGlu5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) attenuated intravenous nicotine self-administration in rats. However, the neural circuitry mediating the action of these systemically administered drugs is not known. Systemic administration of nicotine increases glutamate levels in the nucleus accumbens (NAcc) shell. Furthermore, direct injections of the metabotropic glutamate 2/3 (mGlu2/3) receptor agonist LY379268 in the NAcc shell attenuated nicotine self-administration behavior in rats. mGlu2/3 receptors are predominantly presynaptic and negatively regulate glutamatergic transmission. Hence, the attenuation of the reinforcing effects of nicotine by administration of the mGlu2/3 receptor agonist was possibly due to decrease in postsynaptic glutamatergic neurotransmission resulting from the activation of mGlu2/3 receptors. Based on these findings, we hypothesized that the mGluR5 antagonist MPEP and the NMDA receptor antagonist LY235959 will reduce the reinforcing effects of nicotine by blocking glutamatergic neurotransmission through either the mGlu5 or the NMDA receptors in the NAcc shell.

Methods: To test this hypothesis, we bilaterally microinjected MPEP (0, 10, 20, 40 µg/0.5 µl/site) or LY235959 (0, 0.1, 1, 10 ng/0.5 µl/site) directly into the NAcc shell using a latin-square within-subjects design in rats self-administering nicotine or food. The effects of these compounds on food self-administration served as a control to determine whether the effects of the compounds were specific to nicotine or could be generalized to other reinforcers. Furthermore, these controls helped rule out effects on other critical processes, such as learning and memory or motor activity which are also required for maintenance of self-administration behavior. Both nicotine and food self-administration were conducted under a fixed ratio (FR) schedule (FR 5 Time Out 20 sec) of reinforcement.

Results: The results indicated that microinjection of MPEP (40 µg/0.5 µl/site) in the NAcc shell decreased nicotine self-administration ($n = 8$), but had no effect on food self-administration ($n = 8$). In contrast, LY235959 microinjection in the NAcc shell (10 ng/0.5 µl/site) increased nicotine self-administration ($n = 12$), but had no effect on food responding ($n = 8$), although a non-significant decrease in food responding was seen after the highest LY235959 dose.

Discussion: Thus, our findings indicate that blockade of NMDA and mGlu5 receptors in the NAcc shell had opposite effects on nicotine-self-administration. The decrease in nicotine self-administration after microinjection of MPEP in the NAcc shell indicates that mGlu5 receptor activation is critical for the reinforcing effects of nicotine. On the other hand, the increase in nicotine self-administration after microinjection of LY235959 in the NAcc shell is an interesting finding and suggests either a decrease in the aversive effects of nicotine that may allow increased nicotine intake or an increase in nicotine-seeking behavior. Neither receptor antagonist affected food self-administration, suggesting that activation of either receptor is not required for the reinforcing effects of food, and that the subjects were capable of performing the task after the intracerebral injections. Furthermore, the data suggest that nicotine and food reward may be mediated by

different circuits within the NAcc shell, as has been suggested by previous studies. In conclusion, the results indicate that disruption of glutamatergic transmission in the NAcc shell can have differential effect on nicotine reward depending on the type of glutamatergic receptor that is blocked, thus highlighting the complex interaction of nicotinic receptor activation by exogenously administered nicotine with glutamatergic transmission in the NAcc shell.

Disclosure: M. DSouza: None. A. Markou: Part 1; Abbott GmbH and Company, AstraZeneca, Pfizer, Intracellular Therapeutics, Inc, Lundbeck Research USA, Bristol-Myers Squibb Co., Hoffman-La Roche Inc.. Part 2; University of California San Diego. Part 4; Intracellular Therapeutics, Inc Co., Bristol-Myers Squibb Co., Lundbeck Research USA, Pfizer, AstraZeneca.

208. Adolescent Ethanol Exposure Produces Reductions in ChAT-IR in the Nucleus Basalis and “Depressive-Like” Behavior in Wistar Rats

Cindy Ehlers*, Derek Wills, Wen Liu, Fulton Crews

The Scripps Research Institute, La Jolla, CA

Background: Epidemiological data indicate that excessive alcohol consumption is prevalent among adolescents and may have lasting neurobehavioral consequences including increased risk for the development of alcohol dependence. Sleep difficulties have also been reported to be common in human adolescents and inadequate sleep has been shown to be associated with negative outcomes. Studies from our laboratory, in rats, demonstrate that adolescent ethanol exposure via vapor can produce changes in sleep and arousal, impairments in anxiety and affective behavior as well as cortical, hippocampal, and basal forebrain neurophysiological function, well into adulthood.

Methods: In the present study immunohistochemistry for choline acetyltransferase (ChAT) was determined to assess forebrain cholinergic neurons (Ch1-4) including the Nucleus Basalis of Meynert (Ch4). Stereological assessments of - ChAT-IR positive (cell numbers/mm²). Wistar rats were exposed to ethanol vapor 14 hrs/ day for 35 days from P22-P57 (average BAC 163 mg%). Rats were withdrawn from vapor and assessed for locomotor activity, startle response and immobility in the swim test as adults. Rats were sacrificed at day 72 and perfused for histochemical analyses.

Results: Alcohol exposed rats displayed a significant reduction in the amplitude of their responses to prepulse stimuli during the startle paradigm, had increased locomotor activity in the open field and in locomotor assessment apparatuses, and decreased latency to immobility in the swim test. Quantitative analyses of ChAT immunoreactivity revealed a significant reduction in cell counts in the Ch1-2 and Ch-4 regions of the basal forebrain.

Discussion: These studies demonstrate that behavioral measures of arousal and affective state and ChAT-IR are significantly impacted by chronic adolescence ethanol exposure and withdrawal in Wistar rats. Adolescent ethanol induced loss of ChAT could underlie persistent changes in adult neurophysiology (supported by AAo19969, AAo20022).

Disclosure: C. Ehlers: None. D. Wills: None. W. Liu: None. F. Crews: None.

209. Sustained Triple Reuptake Inhibition Decreases the Firing Activity of Norepinephrine but not Serotonin Neurons in the Rat Brain

Jojo Jiang, Mostafa El Mansari*, Pierre Blier

University of Ottawa Institute of Mental Health Research, Ottawa, ON, Canada

Background: Selective serotonin reuptake inhibitors (SSRIs) is the most frequently used treatment for depression. However, recent studies have established the existence of reciprocal relationships between the serotonin (5-HT), norepinephrine (NE) and dopamine (DA) systems in the brain.¹ Thus, acting on one monoaminergic

system may reverberate on the other two and all three systems must thus be considered when investigating the mechanism of action of antidepressant drugs. As such, a recent approach in the therapy of depression is the development of triple reuptake inhibitors. Given that triple reuptake inhibitors are still being developed and not readily available, administration of escitalopram (an SSRI) plus nomifensine (a NE/DA reuptake inhibitor)² was used to mimic the effect of triple reuptake inhibitors. This combination treatment will be referred to as a ‘triple reuptake inhibitor’. This study was conducted to examine the effects of ‘triple reuptake’ administration on locus coeruleus (LC) NE and dorsal raphe nucleus (DRN) 5-HT neurotransmission.

Methods: Subcutaneously implanted minipumps delivered vehicle, escitalopram (10 mg/kg/day), and/or nomifensine (5 mg/kg/day) for 2 and 14 days. *In vivo* electrophysiological recordings were conducted in anesthetized male Sprague-Dawley rats.

Results: The ‘triple reuptake inhibitor’ decreased the firing of NE neurons by about 60% after both 2- and 14-day administration. A 2-day administration of ‘triple reuptake inhibitor’ resulted in serotonergic firing rates that were the same as control levels. In order to determine if this triple reuptake inhibition produced an increased activation of the 5-HT_{1A} autoreceptor, the selective 5-HT_{1A} antagonist WAY-100635 was injected intravenously; this led a tripling of the firing rate of 5-HT neurons above their normal rate. The firing rate of the 5-HT neurons also remained at control levels after 14 days of ‘triple reuptake inhibition’.

Discussion: As with SSRIs, serotonin/norepinephrine reuptake inhibitors (SNRIs), and selective norepinephrine reuptake inhibitors, the ‘triple reuptake inhibitor’ was shown to decrease the firing rate of LC NE neurons after both 2- and 14-day administration. However, the normal spontaneous firing rate of DRN 5-HT neurons was maintained after the 2-day ‘triple reuptake’ administration compared to the robust inhibitory action of SSRIs. This result suggests that there was an increased activation of the excitatory α_1 -adrenergic and D2 receptors on 5-HT neurons resulting from NE and DA reuptake inhibition, respectively. These results indicate that at optimal triple reuptake inhibition there may be a more rapid enhancing action on 5-HT transmission than with a SSRI alone. Further experiments are necessary to determine the net effect of triple reuptake inhibition on 5-HT, NE and DA transmission. 1. Guiard et al, Int J Neuropsychopharmacol 11: 625-9, 2008 2. Katz et al, J Psychopharmacol 24: 1223-35, 2010.

Disclosure: J. Jiang: None. M. El Mansari: None. P. Blier: Part 2; Wyeth. Part 3; Steel Beach Productions. Part 4; Bristol Myers Squibb, Janssen, Wyeth, Astra Zeneca, Schering-Plough, Labopharm.

210. Psychostimulant Administration Throughout Development

Differentially Alters Dopaminergic Neuronal Function

Annette Fleckenstein*, Lisa McFadden, Paula Vieira-Brock, Amanda Hoonakker, Jonathan Ellis, Scott Allen, Shannon Nielsen, Mario Alburges, Diana Wilkins, Glen Hanson

University of Utah, Salt Lake City, UT

Background: Stimulant abuse among adolescence is an important societal problem. Accordingly, the purpose of the present study is to determine the impact of exposure to two important stimulants, nicotine and methamphetamine, on dopaminergic neuronal function during the transition from adolescence to young adulthood as assessed in a rodent model.

Methods: Male Sprague Dawley rats received nicotine, methamphetamine or vehicle throughout development, beginning in “adolescence” (post-natal day (PND) 40) until “young adulthood” (PND 90). Dopaminergic neuronal integrity was assessed in rat striatum by measuring dopamine transporter immunoreactivity and function using western blotting and [³H]dopamine uptake, respectively. Stimulant levels were assessed using liquid-chromatography-electrospray ionization-tandem mass spectrometry.

Results: Parenteral administration of methamphetamine *per se* from PND 40–90 was without effect on dopaminergic neuronal integrity. However, this same treatment attenuated the persistent decreases in dopaminergic neuronal integrity afforded by a subsequent, repeated high-dose methamphetamine “challenge” administration as assessed 7 d following this neurotoxic methamphetamine treatment. Prevention of methamphetamine-induced hyperthermia contributed to the neuroprotection afforded by stimulant pretreatment. Similarly, pretreatment with nicotine *per se* throughout development was without effect on dopaminergic neuronal function, but attenuated the persistent dopaminergic deficits caused by a subsequent methamphetamine treatment. Unlike methamphetamine pretreatment, hyperthermia was not the sole cause of this protection.

Discussion: Psychostimulant treatment throughout development alters the response to a subsequent neurotoxic stimulant treatment, albeit via different mechanisms. The role of pharmacokinetics in this neuroprotection will be discussed (supported by DA00869, DA13367, DA11389, DA000378 and DA019447).

Disclosure: A. Fleckenstein: None. L. McFadden: None. P. Vieira-Brock: None. A. Hoonakker: None. J. Ellis: None. S. Allen: None. S. Nielsen: None. M. Alburges: None. D. Wilkins: None. G. Hanson: None.

211. Interaction between the Basolateral Amygdala and Dorsal Hippocampus is Necessary for the Reconsolidation of Instrumental Cocaine Memories that Control Drug Context-induce Cocaine-Seeking Behavior in Rats

Audrey Wells, Donna Ramirez, Kate Cowhey, Xiaohu Xie, Heather Lasseter, Rita Fuchs*

University of North Carolina, Chapel Hill, NC

Background: Exposure to a cocaine-associated environmental context can elicit craving and relapse in cocaine users and drug-seeking behavior in laboratory animals. It has been theorized that the memories of context-response-cocaine associations become labile upon retrieval and continued contextual control over drug-seeking behavior depends on the reconsolidation of these associations into long-term memory. Previous studies from our laboratory have shown that anisomycin-sensitive processes in the basolateral amygdala and sodium channel-mediated, but not anisomycin-sensitive, processes in the dorsal hippocampus are necessary for the ability of reconsolidated cocaine memories to guide drug context-induced reinstatement of extinguished cocaine-seeking behavior (Fuchs et al., 2009; Ramirez et al., 2009). The present study evaluated whether the same or different neural mechanisms are required for the processing of reconsolidated cocaine memories as a function of the remoteness of these memories. Using the disconnection procedure, this study also explored whether these brain regions exhibit sequential information processing or mediate memory reconsolidation independently, via parallel circuits.

Methods: Rats were trained to press a lever for un-signaled cocaine infusions in a distinct environmental context. Self-administration training was followed by daily extinction sessions in a distinctly different environmental context. On post-cocaine day 8, the rats were exposed to the cocaine-associated context or a novel unpaired context in the absence of cocaine reinforcement for 15 min. Immediately after this memory reactivation session, the protein synthesis inhibitor anisomycin (ANI; 0 or 125 microg/microl; 0.5 microl/side) or the sodium channel blocker tetrodotoxin (TTX; 0 or 10 ng/microl; 0.5 microl/side) was microinfused bilaterally into the basolateral amygdala or dorsal hippocampus, respectively. In the disconnection experiment, anisomycin was microinfused unilaterally into the basolateral amygdala plus the GABA agonists baclofen/muscimol (0/0 or 1/0.1 mM cocktail; 0.5 microl/side) were microinfused into the contralateral or ipsilateral dorsal hippocampus. The effects of these manipulations were assessed on reinstatement of cocaine-seeking behavior (i.e., non-reinforced lever presses in the cocaine-associated context) after a 24-h (recent memory condition) or 21-d drug-free period (remote memory condition) and at least two additional extinction sessions.

Results: Exposure to the cocaine-associated context reinstated extinguished cocaine-seeking behavior in vehicle-pretreated rats. Tetrodotoxin-induced bilateral neural inactivation of the dorsal hippocampus following cocaine memory reactivation subsequently impaired context-induced cocaine-seeking behavior guided by recent, but not remote, cocaine memories. In contrast, anisomycin treatment in the basolateral amygdala following cocaine memory reactivation subsequently impaired this behavior regardless of memory remoteness. Basolateral amygdala - dorsal hippocampus disconnection (i.e., contralateral anisomycin - baclofen/muscimol manipulation) selectively disrupted context-induced cocaine-seeking behavior guided by recent cocaine memories, whereas the ipsilateral control manipulation failed to alter this behavior. Consistent with memory reactivation-dependent deficits in memory reconsolidation, reinstatement was not impaired in rats that received anisomycin, tetrodotoxin, and/or baclofen/muscimol treatment after exposure to the novel unpaired context.

Discussion: The present findings indicate that the basolateral and dorsal hippocampus form a serial circuit that is necessary for the successful reconsolidation and utilization of recent cocaine-related memories that maintain drug context-induced cocaine-seeking behavior. Activity within the same circuit or within the dorsal hippocampus appears to be necessary for the utilization of only recently reconsolidated cocaine memories. In contrast, anisomycin-sensitive processes in the basolateral amygdala are required for the utilization of both recent and remote cocaine memories. The most plausible explanation for this pattern of findings is that the basolateral amygdala is a likely site of memory reconsolidation. Furthermore, neural activity in the dorsal hippocampus may be necessary for the establishment of a retrieval link to recently reconsolidated amygdala-dependent memories, whereas a different brain regions may provide access to remotely reconsolidated amygdala-dependent memories.

Disclosure: A. Wells: Part 1; National Institute on Drug Abuse. D. Ramirez: National Institute on Drug Abuse. K. Cowhey: None. X. Xie: National Institute on Drug Abuse. H. Lasseter: National Institute on Drug Abuse. R. Fuchs: National Institute on Drug Abuse.

212. Concomitant NET Inhibition Enhances the Antipsychotic-Like Effect of Quetiapine in Rats and Enhances Prefrontal Dopamine Output and Cortical NMDA Receptor-Mediated Transmission

Torgny Svensson*, Carl Bjorkholm, Kent Jardemark, Bjorn Schilstrom, Svante Nyberg, Monica Marcus

Karolinska Institutet, Stockholm, Sweden

Background: Quetiapine is a second-generation antipsychotic drug with a chemical structure similar to clozapine and olanzapine. Like clozapine, quetiapine alleviates both positive and negative symptoms as well as certain cognitive impairments in schizophrenia despite a relatively low D₂ receptor occupancy. Significantly, and distinctively from olanzapine, quetiapine has also shown efficacy as monotherapy in bipolar depression and unipolar depression. Recently, quetiapine has been found to generate a major human metabolite, norquetiapine, which has a different pharmacological profile compared with its parent compound and is a potent norepinephrine transporter (NET) inhibitor. Clinical studies show that reboxetine, a selective NET inhibitor, not only exerts an antidepressant effect but also may improve cognition. We have previously shown in rats that reboxetine potentiates the antipsychotic-like effect of low doses of both raclopride and olanzapine, and concomitantly augments dopamine output as well as NMDA receptor-mediated transmission in the prefrontal cortex, effects that are shared with clozapine, but not typical neuroleptics, and may serve to enhance cognition.

Methods: Since quetiapine is not metabolized to norquetiapine in rodents, we here investigated, in rats, the effects of adjunct NET inhibition by administration of reboxetine and quetiapine separately and in combination on antipsychotic-like activity, using the conditioned avoidance response (CAR) test, dopamine output in the medial prefrontal cortex, using microdialysis in freely moving animals, and cortical NMDA receptor-mediated transmission, using intracellular

electrophysiological recordings in pyramidal cells in a slice preparation.

Results: Reboxetine (6 mg/kg i.p.) potentiated the suppression of CAR by low-dose quetiapine (3 mg/kg i.v.) at 5 and 30 minutes after administration. In analogous biochemical experiments, adjunctive reboxetine treatment also dramatically enhanced the quetiapine-induced prefrontal dopamine output. Moreover, a low, clinically relevant concentration of reboxetine (20 nM) significantly potentiated the effect of a submaximal concentration of quetiapine on NMDA-induced currents in pyramidal cells.

Discussion: The present results propose that concomitant NET inhibition by norquetiapine may not only contribute to the clinical antidepressant effect of quetiapine, but also help maintain its antipsychotic effect in spite of a relatively low level of D₂ blockade. Clinical data show that selective blockage of the NMDA receptor by low, nonpsychotic doses of ketamine causes a specific impairment of verbal working memory, a type of cognitive dysfunction that is frequently encountered both in schizophrenia and major depression. Moreover, recent post mortem studies demonstrate a reduced expression in cortical areas of several NMDA receptor subunits, including NR1 and NR2A, in both schizophrenia and bipolar disorder as well as in major depression. In addition, clinical as well as preclinical studies propose an impaired prefrontal dopamine projection in schizophrenia. Consequently, the enhanced facilitation of prefrontal dopamine output and cortical NMDA receptor-mediated transmission produced by quetiapine combined with a NET inhibitor may contribute to its effectiveness in schizophrenia, bipolar disorder, and major depression as well as to its cognitive enhancing effect.

Disclosure: T. Svensson: Part 1; Lundbeck A/S, Merck, AstraZeneca, Schering-Plough, BiRDs Pharma GmBH, AstraZeneca, J&J, IPAB. Part 4; Schering-Plough, BiRDs Pharma GmBH, AstraZeneca, J & J.

C. Bjorkholm: None. K. Jardemark: None. B. Schilstrom: None. S. Nyberg: Part 5; AstraZeneca. M. Marcus: None.

213. GABAB Receptors Modulate NMDA Receptor Calcium Signals In Dendritic Spines

Jason Chalifoux*, Adam Carter

New York University, New York, NY

Background: Metabotropic GABAB receptors play a fundamental role in modulating the excitability of neurons and circuits throughout the brain. These receptors influence synaptic transmission by inhibiting presynaptic release or activating postsynaptic potassium channels. However, their ability to directly influence different types of postsynaptic glutamate receptors remains unresolved.

Methods: Here we examine GABAB receptor modulation in layer 2/3 pyramidal neurons from the mouse prefrontal cortex. We use two-photon laser-scanning microscopy to study synaptic modulation at individual dendritic spines.

Results: Using two photon optical quantal analysis, we first demonstrate robust presynaptic modulation of multivesicular release at single synapses. Using two-photon glutamate uncaging, we then reveal that GABAB receptors strongly inhibit NMDA receptor calcium signals. This postsynaptic modulation occurs via the PKA pathway and does not affect synaptic currents mediated by AMPA or NMDA receptors.

Discussion: This novel form of GABAB receptor modulation has widespread implications for the control of calcium dependent neuronal function.

Disclosure: J. Chalifoux: None. A. Carter: None.

214. Stress Regulation of Kappa Opioid Receptor Signaling in the Extended Amygdala

Thomas Kash*, Chia Li, Ana Jijon

UNC Chapel Hill, Chapel Hill, NC

Background: Endogenous stress and anti-stress systems co-exist in mammalian organisms. Chronic exposure to stress is hypothesized to

disrupt the relative balance of these opposing systems within key circuitry in the brain, leading to dysregulated emotional behaviors. The kappa opioid receptor (KOP) and its endogenous agonist, the neuropeptide dynorphin, are a critical component of the 'stress' system. Interestingly, dynorphin is expressed in the cell bodies and terminals of the bed nucleus of the stria terminalis (BNST), a brain region associated with anxiety and stress. This suggests that KOP activation in this region may play a role in the regulation of emotional behaviors. However, the impact of KOP activation on synaptic transmission in this region or whether this transmitter system is influenced by stress has not been characterized.

Methods: Using a combination of whole-cell voltage clamp recordings in an *ex vivo* mouse brain slice preparation we investigated the mechanism and impact of KOP activation on inhibitory transmission in the BNST. We then used a combination of electrophysiology and immunohistochemistry to examine the impact of a well characterized stress paradigm in both C57BL/6J and DBA/2J mice. We selected these particular strains, as previous work has demonstrated that they exhibit divergent changes in anxiety like behavior following stress exposure.

Results: We found that activation of KOP reduced GABAergic transmission through a presynaptic mechanism. We next examined if this form of modulation was influenced by genetic variation and a history of stress exposure. We found that the inhibitory effect of KOP activation on synaptic inhibition was significantly greater in DBA/2J mice compared to C57BL/6J mice. Further, we found that chronic, but not acute restraint, altered KOP modulation in C57BL/6J mice; while both acute and chronic restraint altered KOP modulation in DBA/2J mice.

Discussion: These findings suggest that genetic variance can influence activation of neuronal circuitry governing the stress response.

Disclosure: T. Kash: None. C. Li: None. A. Jijon: None.

215. Decreased Number of Somatostatin and Parvalbumin-Positive Hippocampal Interneurons in Schizophrenia

Christine Konradi*, Eric Zimmermann, C. Kevin Yang, Kathryn Lohmann, Paul Gresch, Harry Pantazopoulos, Sabina Berretta, Stephan Heckers

Vanderbilt University Medical Center, Nashville, TN

Background: Schizophrenia is associated with abnormal hippocampal structure and function.

Methods: We counted hippocampal cells in schizophrenia patients and control subjects throughout the entire cornu ammonis, using a uniformly random sampling strategy, and examined the density of interneurons expressing the markers somatostatin and parvalbumin. Human hippocampi of 14 schizophrenia patients and 18 control subjects were used in the study. Tissues were obtained from the Harvard Brain Tissue Resource Center at McLean Hospital. Tissue was cut at 2.5 mm intervals in the coronal plane throughout the rostral-caudal extent of the hippocampus. Sections were either Nissl-stained or immunohistochemically stained with antibodies against somatostatin or parvalbumin protein. Messenger RNA was extracted from fixed tissue and real-time quantitative PCR was performed.

Results: Data were collected on regional volumes of pyramidal and non-pyramidal cell layers, overall neuron size and numbers, number of somatostatin - and parvalbumin positive interneurons and messenger RNA levels of somatostatin, parvalbumin and glutamic acid decarboxylase 1. The ratio of non-pyramidal volume to pyramidal volume was within normal range in the entire dataset, but below normal range when females only were examined. Total neuron number was not significantly different between both experimental groups, though a region x diagnosis effect was observed, with reduced numbers of cells in CA1 and CA4, but not in CA2/3. The number of somatostatin-positive neurons was reduced in all hippocampal sectors (CA1, CA2/3 and CA4), as was the number of parvalbumin-positive neurons in sectors CA1 and CA4, with a trend toward reduction in sector CA2/3 ($p = 0.053$). All data were corrected for age, post-mortem

interval, gender and brain hemisphere. Real-time quantitative PCR confirmed the pathological changes with a downregulation of mRNA molecules for somatostatin and parvalbumin.

Discussion: Our data are the first collected from the entire hippocampus and confirm abnormalities in interneuron populations in schizophrenia.

Disclosure: C. Konradi: None. E. Zimmermann: None. C. Yang: None. K. Lohmann: None. P. Gresch: None. H. Pantazopoulos: None. S. Beretta: None. S. Heckers: None.

216. Diverse Psychotomimetics Produce Prefrontal Dysfunction via Convergent Effects on L-Type Calcium Channels

Vikaas Sohal*, Emily Guhl, Tasha Patel, Karl Deisseroth

Univ of CA San Francisco, San Francisco, CA

Background: It is widely believed that dysfunction of the prefrontal cortex (PFC) underlies many of the most debilitating aspects of schizophrenia, including deficits in attention, working memory, problem solving, and social cognition. These cognitive symptoms are poorly treated by current medications and cause major functional impairment, yet it has not been possible to correlate specific cellular abnormalities with prefrontal dysfunction in schizophrenia. Here we provide evidence for such a link between excessive calcium channel activation, aberrant neural network activity, and schizophrenia-like behaviors in animals.

Methods: We chose to look for cellular correlates of schizophrenia in layer V of the PFC for three reasons. First, the PFC is widely implicated in schizophrenia and the cognitive functions that are disrupted in schizophrenia. Second, all known antipsychotic drugs block the D₂ receptor, and within the PFC, most D₂ receptors are located in layer V. Finally, layer V represents a “critical node” that controls output from PFC. Therefore we studied how two drugs that both produce schizophrenia-like behavior in animals affect layer V neurons in the PFC: Quinpirole, which activates D₂ receptors, and phencyclidine (PCP), which elicits a schizophrenia-like syndrome in humans and has long been used to model schizophrenia in animals.

Results: Surprisingly, although Quinpirole (5–20 uM) and PCP (5 uM) are thought to act via different mechanisms, we found that they both elicit similar abnormal behavior in a subset of layer V prefrontal neurons which could be identified by specific electrophysiological properties. Specifically, we found that in these neurons, both Quinpirole (16/17 cells) and PCP (6/9 cells) elicited an activity-dependent depolarization that could increase spiking or produce depolarization blockade during responses to depolarizing current injection. Moreover, this activity-dependent depolarization was followed by a prolonged afterdepolarization that could produce spiking or even membrane bistability following the cessation of stimulation (amplitude = 7.5 ± 1.8 mV, decay time constant = 126 ± 37 msec). These effects were blocked by Nifedipine (10 uM, n = 3/3 cells in Quinpirole, 2/2 cells in PCP), a specific antagonist for L-type calcium channels, which have been implicated in schizophrenia by genome-wide association studies. Interestingly, the D₂ agonists Haloperidol (0.2–10 uM; 9 cells) and Sulpiride (5 uM; 2 cells) blocked the effects of Quinpirole but not those of PCP. The activity-dependent depolarization and afterdepolarization elicited by Quinpirole reduced the amount of information that layer V prefrontal neurons transmit about input stimuli (n = 8 cells, p < 0.05), demonstrating that this phenomenon could contribute to prefrontal dysfunction in schizophrenia. Because Nifedipine reverses the effects of Quinpirole and PCP, we hypothesized that Nifedipine might alleviate some effects of PCP in behaving mice. PCP (5 mg/kg) causes deficits in social behavior, and consistent with our hypothesis, we found that pre-treating mice with Nifedipine (15 mg/kg) rescues PCP-induced deficits in a dose-dependent manner (p < 0.05). Nifedipine also reduces other psychotic behaviors induced by PCP (e.g. catatonic posturing and stereotyped movements). These results suggest that excessive calcium channel activity may indeed contribute to symptoms of schizophrenia.

Discussion: Although PCP has been assumed to exert its psychotomimetic effects by blocking NMDA receptors, while D₂ agonists such as Quinpirole act via a distinct mechanism, we found that both PCP and Quinpirole produce aberrant activity in a subset of layer V pyramidal neurons via L-type calcium channels. Layer V pyramidal neurons send outputs from the PFC, thus, via this effect, D₂ receptors and L-type calcium channels are well poised to modulate prefrontal output, contributing to normal functions such as corollary discharge as well as pathological output in conditions such as schizophrenia. By linking a specific ion channel in a specific subpopulation of prefrontal neurons to aspects of schizophrenia, our results may serve as a powerful guide for the development of novel therapeutics targeted to specific symptoms of prefrontal dysfunction, or to specific patients with abnormalities in pathways involving voltage-dependent calcium channels. Acknowledgements: Supported by NIMH, IMRHO, NARSAD, Stanford University, HHMI, CIRM, McKnight, Coulter, NSF, NIMH, NIDA, and the Kinetics and Keck Foundations.

Disclosure: V. Sohal: None. E. Guhl: None. T. Patel: None. K. Deisseroth: None.

217. Effects of Methylphenidate on Spike Train Coding of Sensory and Decision-Related Signals in Rodent Thalamic and Cortical Circuits during Quiet Resting and Sustained Attention

Barry Waterhouse*, Kara Agster, Brian Clark, Jed Shumsky

Drexel University College of Medicine, Philadelphia, PA

Background: While the biochemical action of the psychostimulant methylphenidate (MPH) is well established, i.e blockade of catecholamine transmitter reuptake, the physiological basis for the efficacy of this drug in promoting wakefulness and enhancing cognitive function is unknown. The present study assessed the effects of low dose MPH on spike train coding of sensory and decision-related signals in thalamic and cortical circuits of rat brain during quiet resting or performance of a sustained attention task.

Methods: Extracellular recordings using fine wires were made from visual and cognitive brain regions while animals were either quietly resting in a behavioral chamber or performing a visually guided sustained attention task (modified from McGaughy and Sarter, 1995). Task naïve animals were presented with a pseudorandom sequence of light flashes of 10, 15, 25, or 40 ms duration.

Results: Lateral geniculate (LGN) unit activity was monitored following systemic injections of either saline or MPH (0.5, 2, or 5 mg/kg). MPH facilitated LGN unit responses to task-related visual stimuli according to an inverted-U dose response function with optimal response augmentation occurring at 2 mg/kg, a dose known to achieve drug plasma levels that are effective in treating ADHD and in facilitating rat performance in the sustained attention task. Evidence of enhanced responding included increased magnitude of both excitatory and inhibitory components of the response and greater temporal fidelity to stimulus presentation. Recordings from medial prefrontal cortex (PFC) in task-performing animals revealed neurons that respond to light cues, with stronger responses in successful trials. Like LGN cells, the responses of PFC neurons to light cues were also enhanced by 2 mg/kg MPH. Prior studies have shown that MPH-mediated enhancement of performance in the sustained attention task is blocked partially by pre-treatment with the alpha one antagonist, prazosin.

Discussion: Acute administration of MPH produces noradrenergic-like modulatory effects in sensory and cognitive circuits of rat brain. Our working hypothesis is that such physiological effects are at least partially responsible for the drug's ability to enhance vigilance and executive functions in the behaving animal as demonstrated in a rodent model of sustained attention. Support: NIDA DA017960 and PA Dept of Health to BDW and NIMH MH084474 to KLA.

Disclosure: B. Waterhouse: None. K. Agster: None. B. Clark: None. J. Shumsky: None.

218. Do Sleep Items on Depression Scales Correlate with Sleep EEG Measures in Adolescents with MDD?

Rich Dopp, Robert Hoffmann, Taryn Mayes, Graham Emslie,
Roseanne Armitage*

University of Michigan, Ann Arbor, MI

Background: Major depressive disorders (MDD) are strongly associated with sleep complaints in both children and adolescents. Sleep EEG abnormalities confirm subjective sleep complaints, but are more common in depressed adolescents than in younger children. Moreover, sleep disturbance or changes in sleep habits are considered diagnostic features of MDD and individual sleep items are included on most symptom severity scales. However, it is not clear to what degree sleep items from symptom severity scales agree with sleep EEG data. Of particular interest was determining how best to define sleep onset insomnia, and whether the adult definition of sleep onset insomnia of 30 minute sleep latency on 3 nights per week or more was adequate in adolescents with MDD. The purpose of the present study was to assess the relationship among sleep EEG measures, self-reported sleep diary data and sleep items on the Children's Depression Rating Scale (CDR-S), Schedules for Affective Disorders and Schizophrenia for School-Aged Children: Present and Lifetime (K-SADS-PL) in symptomatic, unmedicated adolescents with MDD.

Methods: 48 outpatients diagnosed with MDD, 13-18 years of age, participated in study. A minimum score of 40 on the CDR-S was required for enrollment. Each participant maintained a regularized sleep schedule for 1 week prior to study in the laboratory. Actigraphy and sleep diary data were recorded during the week, followed by 2 consecutive nights of polysomnography in the lab. Night 1 served as laboratory adaptation and baseline EEG measures were collected on night 2. Visual sleep stage scoring was conducted according to standard criteria. Sleep diary measures included: sleep latency, bed and rise-times, total sleep time, number and duration of awakenings and how rested subjects felt upon awakening. Sleep EEG measures included the latency to persistent sleep, total sleep time, sleep efficiency, REM latency and the percentage of sleep spent in Stages 1,2, SW and REM.

Results: Home sleep diary data was a better correlate of sleep EEG measures than the total symptom severity scores or individual sleep items. None of the correlations between sleep EEG measures and symptom severity total score or individual items were significant after Bonferroni correction. By contrast, self reported sleep latency was significantly correlated with EEG-derived sleep latency, % light, Stage 1 sleep, and sleep efficiency accounting for more than 60% of the overall variance by regression analysis. Moreover, those who reported the longest sleep latency by diary had the worst sleep in the lab. Reporting taking 25 minutes or longer to fall asleep by diary was the optimal cut-point for identifying more disturbed sleep EEG.

Discussion: Neither CDR-S nor KSADS-PL sleep items were good correlates of sleep EEG measures suggesting that while adolescents with MDD may endorse sleep complaints during clinical interview, these items do not identify those most likely to show sleep EEG-defined abnormalities. Home sleep diaries are better correlates of sleep EEG-defined sleep latency and identify those adolescents most likely to show classic sleep EEG abnormalities associated with depression. The findings of this study are relevant to identifying which adolescents with MDD will be most likely to show prolonged sleep latency and abnormal sleep architecture in the laboratory and as such, may be an important screening tool for sleep research studies. Sleep diaries may also be of clinical use in quantifying sleep complaints in depressed adolescents.

Disclosure: R. Dopp: None. R. Hoffmann: None. T. Mayes: None. G. Emslie: Part 4; Eli Lilly, Forest Laboratories, Glaxo Smith Kline, Wyeth-Ayerst, Shire, BioBehavioral Diagnostics. R. Armitage: Part 1; Esai Pharmaceuticals.

219. Multi-site Unit Recordings In Rat Medial Prefrontal Cortex During Cocaine Self-administration, Extinction and Reinstatement

David Moorman, Gary Aston-Jones*

Medical University of South Carolina, Charleston, SC

Background: The prefrontal cortex (PFC) is critical for goal-directed, reward-seeking behaviors, and is crucial for reinstatement of extinguished drug-seeking. In human addicts, drug-associated cues activate PFC neurons, and PFC-associated cognitive deficits are frequently observed. In rodent, inhibition or activation of discrete regions of medial PFC (mPFC) produces strong control over drug-seeking. Despite this robust association, little is known about how mPFC neuronal activity relates to drug-seeking. Previous studies investigated the activation of mPFC neurons during drug self-administration (SA). However, because the mPFC is involved in many components of reward-seeking behavior, we sought to characterize the relationship of mPFC neuronal activation to multiple aspects of drug abuse.

Method: We recorded from neurons in mPFC of Sprague Dawley male rats during cocaine SA, extinction (EXT), and reinstatement. During SA, rats pressed an active lever for intravenous cocaine (FR-1; 0.2 mg/50 µL infusion; 20-s TO; 2-hr sessions) and a discrete tone-light cue. Presses on the inactive lever were recorded but produced no outcome. After two weeks of stable SA (>10 presses/session), animals were tested in a combined EXT/SA session (1-hr each). Animals then underwent EXT sessions in which lever presses were recorded but produced no outcome. Following at least two days of extinguished behavior (<10 presses/session) animals underwent cue-induced reinstatement sessions in which active lever-presses produced tone-light cues but no delivery of cocaine. Single-neuron recordings were made from microwire arrays implanted bilaterally in prelimbic (PL) and infralimbic (IL) areas of the mPFC during each of the behavioral stages. One goal of these studies was to compare activities in these two PFC regions during SA and EXT.

Results: Preliminary results from 12 animals demonstrated robust differences in neuronal activity across the behavioral stages. Initial analyses of neural activity in the combined EXT/SA session produced two major findings. First, overall activity was dramatically different in each of the two conditions with a rapid transition occurring at the switch from EXT to SA. Intriguingly, these transitions were not uniformly oriented. In many cases activity transitioned from low to high in neurons recorded on some wires but displayed the opposite effect in neurons recorded on other wires in the same rat. Second, bursts of activity related to lever-press and/or cue presentation were substantially different in the two conditions, with responses primarily present during the SA sessions.

Discussion: Overall, these results demonstrate a robust difference in mPFC activation during drug-seeking in the presence vs. absence of drug reward. Further analyses of these data, as well as results collected during reinstatement sessions, is currently underway and will provide a more thorough understanding of how drug-seeking behavior is encoded by prefrontal neurons. Supported by PHS grant R37 06214 and P50 DA015369.

Disclosure: D. Moorman: None. G. Aston Jones: Part 1; Sanofi Aventis, Lundbeck Pharmaceuticals, Lilly Pharmaceuticals. Part 4; Lilly Pharmaceuticals, Cephalon.

220. The mGluR7 Allosteric agonist AMN082 produces Antidepressant-Like Effects by Modulating AMPA and NMDA Receptors

Stefania Bradley*, Jason Uslaner, Rose Flick, Ariel Lee, Kristina Groover, Lisa Gold, Peter Hutson

Merck and Co., Inc., West Point, PA

Background: Commonly prescribed antidepressants affect the reuptake and/or metabolism of biogenic amines. However, in well-

controlled clinical trials these antidepressants take several weeks of treatment to produce significant symptom remission. Recently, promising results show that ketamine, a dissociative anesthetic agent that noncompetitively antagonizes NMDA receptors, appears to have a rapid antidepressant effect at sub-anesthetic doses in clinically depressed patients. These preliminary results indicate that modulation of the glutamatergic system could be an efficient way to achieve antidepressant activity.

Methods: Metabotropic glutamate receptor (mGluR) ligands seem to be promising agents to treat several central nervous system disorders, including psychiatric diseases. Several investigators have reported the potential antidepressant-like activity of the first, selective, and bioavailable mGluR₇ agonist, AMN082 (N,N'-dibenzhydryl-ethane-1,2-diamine dihydrochloride). The activity of AMN082 has been previously evaluated in several animal models of "behavioral despair" (forced swim test and in tail-suspension test) commonly used to identify clinically useful antidepressants. We extended the characterization of AMN082 by investigating the potential mechanisms of action by which AMN082 exerts its antidepressant-like activity.

Results: Our behavioral studies demonstrate that AMN082-induced decreased immobility in the tail suspension test is reversed by the selective AMPA receptor antagonist NBQX. In contrast, NBQX failed to reverse the effects of imipramine in the same behavioral model. We have also investigated the underlying physiological mechanisms of action by exploring the involvement of glutamate signaling. We have observed that behaviorally efficacious doses of AMN082 induce phosphorylation of AMPA and NMDA receptor subunits in the hippocampus and striatum.

Discussion: These results confirm that mGluR₇ could represent a novel target for treating depression and suggest that the antidepressant-like effects of AMN082 might be due to its ability to modulate AMPA and NMDA receptors activity.

Disclosure: S. Bradley: Part 5; Merck and Co., Inc. J. Uslaner: Merck and Co., Inc. R. Flick: Merck and Co., Inc. A. Lee: Merck and Co., Inc. K. Groover: Merck and Co., Inc. L. Gold: Merck and Co., Inc. P. Hutson: Merck and Co., Inc.

221. Shifting Topographic Activation and 5-HT_{1A}-Receptor Mediated Inhibition of Dorsal Raphe Serotonin Neurons Produced by Nicotine Exposure and Withdrawal

Kathryn Commons*, Robin Sperling

Children's Hosp/Harvard Medical School, Boston, MA

Background: Nicotine administration activates serotonin (5-HT) neurons innervating the forebrain and this is thought to reduce anxiety. Nicotine withdrawal has also been associated with an activation of 5-HT neurotransmission, although withdrawal increases anxiety. In each case, 5-HT_{1A} receptors have been implicated in the response.

Methods: To determine if there are different subgroups of 5-HT cells activated during nicotine administration and withdrawal, we mapped the appearance of Fos, a marker of activation, in 5-HT cells of the dorsal and median raphe nuclei (DR and MR). To understand the role 5-HT_{1A} receptor-mediated inhibition of 5-HT cell activity during these conditions, we administered a selective 5-HT_{1A}-receptor antagonist and measured novel disinhibited Fos expression within 5-HT cells.

Results: Using these approaches, we found evidence that acute nicotine activates 5-HT neurons rostral-dorsal and in the lateral wings of the DR and produces a 5-HT_{1A} dependent inhibition of cells located ventrally both at rostral and mid levels. Acute nicotine given to animals with previous chronic nicotine exposure continued to activate Fos expression in a similar pattern, predominantly rostral in the DR, and this was accompanied by a novel 5-HT_{1A}-mediated inhibitory effect in the caudal DR. This pattern nearly reversed during nicotine withdrawal when there was evidence for caudal activation and mid-and rostral-5-HT_{1A}-dependent inhibition.

Discussion: These results suggest that the distinct behavioral states produced by nicotine exposure and withdrawal correlate with

reciprocal rostral-caudal patterns of activation and 5-HT_{1A}-mediated inhibition of DR 5-HT neurons. The complimentary patterns of activation and inhibition suggest that 5-HT_{1A} receptors may help shape distinct topographic patterns of activation within the DR under these conditions.

Disclosure: K. Commons: None. R. Sperling: None.

222. Projections from the Infralimbic Cortex and the Ventral Tegmental Area Compete in the Nucleus Accumbens Shell to Regulate Cocaine-Seeking Behavior in Rats

Ryan Lalumiere*, Peter Kalivas

University of Iowa, Iowa City, IA

Background: The infralimbic cortex (IL), nucleus accumbens (NA) shell, and ventral tegmental area (VTA) regulate cocaine-seeking behavior. While the IL and VTA both project to the NAshell, evidence indicates that the IL and VTA oppositely regulate cocaine-seeking. Specifically, activity in the IL suppresses, whereas activity in the VTA promotes, drug-seeking behavior. Recent studies with regard to the NAshell, however, have been conflicting, as evidence indicates that NAshell activity is involved in both the suppression and reinstatement of drug-seeking. Together, the findings suggest that the NAshell promotes or suppresses drug-seeking behavior depending on the activity of structures that project to the NAshell. Thus, it is hypothesized that, when dopaminergic inputs from the VTA are activated during a reinstatement session, drug-seeking is promoted. In contrast, when glutamatergic inputs from the IL are activated, drug-seeking is suppressed. The present experiments addressed this hypothesis.

Methods: Male Sprague-Dawley rats (225-250 g) underwent surgeries for implantation of an intravenous catheter, a double-barreled cannula aimed at the IL, and bilateral cannulas aimed at the VTA or NAshell. Rats underwent two weeks of cocaine self-administration (2 hr sessions/day) in which cocaine infusions were paired with a light and tone, and then extinction training (2 hr/day) for a minimum of 12 days. Rats then underwent cue-induced reinstatement. Immediately prior to the reinstatement session, rats received intra-IL microinjections of the allosteric AMPA receptor potentiator PEPA (30 ng) or vehicle. In addition, they also received concurrent microinjections into the NAshell of dopamine (20 µg) or vehicle or the AMPA receptor antagonist CNQX (1 nmol) or vehicle or into the VTA of the µ-opioid receptor agonist DAMGO (0.1 nmol) or vehicle.

Results: Those rats receiving PEPA had decreased active lever presses during the reinstatement session. Concurrent intra-VTA microinjections of the µ-opioid receptor agonist DAMGO, which disinhibits VTA dopamine neurons, reversed the effects of PEPA and restored cue-induced drug-seeking. Similarly, intra-NAshell microinjections of dopamine reversed the effects of PEPA and restored drug-seeking behavior. Intra-NAshell microinjections of the AMPA receptor antagonist CNQX also reversed the effects of PEPA microinjections into the IL and restored cue-induced drug-seeking.

Discussion: These findings indicate that activation of glutamatergic AMPA receptors or dopaminergic receptors in the NAshell differentially affect drug-seeking behavior in rats. Combined with previous findings, the present results suggest that IL projections to the NAshell and VTA projections to the NAshell compete in the shell to suppress and promote, respectively, drug-seeking behavior.

Disclosure: R. LaLumiere: None. P. Kalivas: None.

223. Glutamatergic Regulation of Raphe Neurons is Dependent on the Activity of Presynaptic Inputs and the Unique Morphology of Lateral Wing 5-HT Neurons

LaTasha Crawford*, Caryne Craige, Sheryl Beck

Children's Hospital of Philadelphia Research Institute, Philadelphia, PA

Background: Characterizing glutamatergic input to dorsal raphe (DR) 5-HT neurons is crucial for our understanding of how the glutamate

and serotonin systems interact in the healthy brain and may be altered in psychiatric disorders such as anxiety, depression, and schizophrenia. The markers of glutamatergic terminals, vGlut1, 2, and 3, reflect inputs from regions such as the mPFC, lateral habenula, or local inputs from within the DR, respectively. Each of the three markers exhibits a unique pattern throughout the ventromedial (vmDR) and lateral wing (lwDR) subregions of the DR, though the contribution of glutamatergic input to 5-HT neurons in these subregions is unclear.

Methods: Adult male mice were used at 8 to 16 weeks of age and were from a line of mice whose 5-HT neurons express yellow fluorescent protein, enabling visualization of 5-HT neurons during electrophysiology recordings in brain slices. Immunohistochemical detection of vGlut protein was performed using wild type littermates from the same line of mice. In brief, antibodies recognizing vGlut 1, 2, and 3 were used to map the distributions of glutamate terminals among tryptophan hydroxylase (TPH)-labeled 5-HT neurons in the vmDR and lwDR subregions. Electrophysiology was used to measure the functional correlates of glutamate input to DR neurons. Mice were sacrificed and their brains dissected rapidly to generate 200 micron-thick slices (Beck et al. 2004, Lemos et al. 2006, Crawford et al. 2010). Visualized whole-cell patch clamp recordings measured AMPA receptor mediated spontaneous excitatory post-synaptic currents (sEPSC), and miniature excitatory post-synaptic currents (mEPSC). The 5-HT identity and DR subregion of recorded cells were confirmed using immunohistochemical detection of TPH and biocytin. Cells filled with biocytin during recordings were imaged using confocal microscopy; soma and dendrite morphology were analyzed using Neurolucida software.

Results: We confirmed that in the mouse, punctate staining of vGlut2 was homogenous throughout the DR subregions while vGlut1 and vGlut3 demonstrated a slight decrease in density within areas where lwDR 5-HT neurons were found. Whole-cell patch clamp recordings of sEPSC activity revealed differences in glutamatergic input to lwDR 5-HT neurons compared to vmDR neurons. Despite the equal or decreased density of various types of glutamate terminals, electrophysiological recordings revealed that lwDR 5-HT neurons had an increased frequency of sEPSCs. The increased sEPSC frequency was not explained by an increase in the number of glutamate terminals or likelihood of glutamate release because the frequency of action potential-independent mEPSC events was decreased in lwDR neurons when compared to vmDR neurons. This suggests that the lwDR neurons receive increased local glutamate input from within the midbrain slice preparation. Because the dendritic morphology of DR 5-HT neurons has never been quantified, it was unknown whether differences in sEPSC input might be due to differences in dendritic structure. The morphology of lwDR 5-HT neurons differed from that of vmDR neurons, including larger cell somas and longer dendrites with more extensive branching. The differences between vmDR and lwDR dendrite number and dendrite length were specific for 2nd and 3rd order branches. While increased sEPSC frequency was correlated with increased mean length of 2nd order branches in all recorded cells, this relationship was predominately seen in vmDR neurons and was not present when lwDR neurons were grouped separately. Thus, the increased dendritic complexity cannot fully explain the increased frequency of sEPSCs in lwDR neurons, as there were several lwDR cells for which this relationship did not hold true.

Discussion: Collectively, these data suggest that spontaneous glutamatergic input in the DR is the result of selective innervation of specific subpopulations of 5-HT neurons and is heightened in the lwDR. These data imply that the communication within the midbrain selectively controls output from the exclusive projections of subfields of the raphe. The glutamatergic input and morphology of lwDR neurons likely synergizes with previously described distinctions in membrane characteristics (Crawford et al., 2010) to regulate 5-HT output in midbrain and hindbrain regions responsible for sympathomotor responses and panic. Subregional differences in glutamate modulation of 5-HT neuron activity may thereby result in specific, diverse effects on 5-HT output in limbic brain regions and brainstem

centers responsible for the behavioral and physiological components of anxiety and other psychiatric disorders.

Disclosure: L. Crawford: Part 4; Pfizer Animal Health scholarship funds my education in veterinary school as of 9-1-10 at the Univ of PA School of Veterinary Medicine. All research presented here was carried out prior to scholarship. C. Craigie: None. S. Beck: None.

224. The Neural Circuitry of Conditioned Fear Extinction in Obsessive Compulsive Disorder

Mohammed Milad*, Mohamed Zeidan, Clas Linnman, Jennifer Greenberg, Eric Jenike, Martha Falkenstein, Roger Pitman, Michael Jenike, Scott Rauch, Sabine Wilhelm

Massachusetts General Hospital & Harvard Medical School, Charlestown, MA

Background: Human neuroimaging studies implicate the medial orbitofrontal cortex (mOFC) and ventromedial prefrontal cortex (vmPFC) in fear extinction_prefrontal regions also known to be dysfunctional in patients with anxiety disorders, such as obsessive compulsive disorder (OCD). Several studies have examined the role of these prefrontal regions using neurocognitive or symptom-provocation paradigms. However, direct tests of their functional integrity during fear extinction remain to be conducted.

Methods: To examine this circuit, we used a two-day fear conditioning procedure on OCD patients and healthy controls while in an fMRI scanner. Skin conductance response was monitored as a behavioral index of conditioning. On day 1, subjects underwent conditioning and extinction. On day 2, extinction recall (memory) was assessed.

Results: Our preliminary psychophysiological results indicate that OCD patients showed intact fear acquisition and extinction training on day 1, but exaggerated fear responses relative to controls on day 2 suggesting impaired recall of the extinction (safety) memory. During extinction recall, fMRI analysis revealed greater activation in the hippocampus and amygdala in OCD patients relative to controls_regions involved in fear expression. Furthermore, OCD patients displayed a failure to activate the vmPFC during extinction training and extinction recall_the latter of which is similar to brain dysfunction observed in PTSD patients.

Discussion: Collectively, our data suggest that OCD patients appear to exhibit impaired extinction recall that is associated with elevated fear responses in the amygdala and a failure to activate the vmPFC.

Disclosure: M. Milad: None. M. Zeidan: None. C. Linnman: None. J. Greenberg: None. E. Jenike: None. M. Falkenstein: None. R. Pitman: None. M. Jenike: None. S. Rauch: None. S. Wilhelm: None.

225. Atypical Brain Function Underlying Sensorimotor Alterations In Autism

Matthew Mosconi*, Stephen Coombes, Lisa Ankeny, Anna-Maria D'Cruz, Zhe Zhang, Lauren Schmitt, Tin Khine, David Vaillancourt, John Sweeney

University of Illinois - Chicago, Chicago, IL

Background: Sensorimotor disturbances and dyspraxia are present in the majority of individuals with autism, yet the brain system abnormalities underlying these deficits are not yet well established.

Methods: Using functional MRI, we studied 16 individuals with autism and 17 age-matched healthy control individuals performing a sustained precision grip force task. All subjects were right-handed. They viewed a white force bar that moved upwards with increased force toward a fixed green target bar. Subjects were instructed to press on a force transducer in order to maintain the white force bar at the level of the green bar by applying a constant force. The green target bar was set to 15% of each individual's maximum force contraction. The gain of the feedback, defined as the vertical distance the white bar moved per Newton of force applied to the transducer, was varied across three levels (low, medium and high) each presented for three

26 sec blocks. When gain was low, the white bar moved a smaller distance for every Newton of force applied compared to when gain was high.

Results: Subjects with autism and controls did not differ on their maximum force level, mean force level or the duration of sustained force. The precision of sustained force was decreased for subjects with autism relative to controls, $F(1,31) = 4.57$, $P < .05$, indicating a difficulty using sensorimotor feedback to sustain a constant force output. This was true especially when the gain setting was low; subjects with autism were less able to sustain precise force levels when visual feedback was less precise, $F(2,30) = 3.18$, $P < .05$. During the low gain condition, subjects with autism showed reduced activation in visuomotor regions, including left motor cortex, left dorsal premotor cortex, bilateral superior parietal lobule, bilateral anterior cerebellum (lobules IV/V) and right posterior cerebellum (lobules V/VI). Within the low gain condition, decreased force precision was also associated with decreased activation within right inferior frontal gyrus, left medial frontal gyrus, anterior cingulate cortex and right lateral cerebellum (Crus I). During the low gain condition, subjects with autism showed increased activation in right MT/V5 and left posterior cerebellum (lobule VI/Crus I). During the high gain condition, subjects with autism showed reduced activation in left middle frontal gyrus, right inferior parietal lobule and bilateral V3. For this condition, they also demonstrated increased activation relative to controls in the following visuomotor regions: bilateral supplementary motor area, left motor cortex, right MT/V5, right anterior cerebellum (lobules IV-VI) and bilateral posterior cerebellum (lobule VII/Crus I). Increased activation within posterior cerebellum was associated with increased force precision during the high gain condition. Also during the high gain condition, subjects with autism showed increased activation within the medial frontal gyrus, right putamen, right caudate and right thalamus.

Discussion: Individuals with autism show reduced steady-state visuomotor precision and dysfunctions within brain systems supporting sensorimotor task performance. Both behavioral and brain alterations differed as a function of gain suggesting that the severity of visuomotor deficits and sensorimotor brain dysfunction in autism are dependent on the precision of sensory feedback. Atypical recruitment of frontostriatal circuitry was observed in subjects with autism during high gain conditions, perhaps as a compensatory reliance on voluntary motor control systems. Decreased activation in visuomotor brain regions during low gain conditions suggests that these systems are especially disrupted when less precise sensory feedback is available to provide online guidance for ongoing motor performance. This modulation of motor output is likely guided by cerebellar systems which appear to be impaired in autism and a major cause of poor fine motor control. While cerebellar pathology has been repeatedly documented in autism, this study provides novel evidence that cerebellar dysfunctions and their interaction with neocortical systems may underlie the dyspraxia that is observed in the majority of individuals with autism.

Disclosure: M. Mosconi: None. S. Coombes: None. L. Ankeny: None. A. D'Cruz: None. Z. Zhang: None. L. Schmitt: None. T. Khine: None. D. Vaillancourt: None. J. Sweeney: Part 1; Pfizer. Part 2; Janssen. Part 4; Janssen.

226. Neural Mechanisms Of Depression Following Left Basal Ganglia Stroke

Sergio Paradiso*, Katharine Ostedgaard, Jatin G. Vaidya, Robert G. Robinson

University of Iowa, Iowa City, IA

Background: Depression following stroke is a highly incapacitating clinical condition affecting about 40% of patients with focal cerebrovascular damage. The mechanisms of post-stroke depression remain poorly understood. Neuroimaging studies have shown that depression without stroke or other gross brain damage is associated with hyperactivity of the amygdala and midline frontal lobe structures

and hypoactivity of dorsal lateral prefrontal cortex. The goal of the present study was to examine the mechanisms of depression resulting from left basal ganglia stroke because stroke victims with damage in the left basal ganglia have been shown to be particularly at risk for depression. Brain activity and depression were measured in patients with stroke in the left basal ganglia and non-brain-damaged volunteers. It was hypothesized that depression severity would positively correlate with brain activity in amygdala and mesial prefrontal regions, while the relation would be inverse for dorsal lateral prefrontal structures.

Methods: Depression was assessed in six patients with stable strokes localized to the left basal ganglia (2 female, 33.3%; mean age = 67.5 ± 9.4 years) and no history of depression prior to stroke and 12 non-depressed healthy volunteers matched for gender and age (4 female, 33.3%; mean age = 65.5 ± 6.3 years). Brain activity was measured while subjects viewed emotionally neutral human facial expressions using positron emission tomography and the [^{15}O]water method. PET activity, excluding the damaged areas, was normalized to an average value of 1 by dividing by the global activity level.

Results: Five (83%) patients with left basal ganglia stroke met DSM-IV criteria for major depressive episode, while none of the non-brain-damaged volunteers did. Depression severity on the Hamilton Depression Scale among left basal ganglia subjects was 16.0 (± 6.8) while in healthy volunteers was 3.8 (2.7) (Mann-Whitney $U = 4.5$, $p < .01$). A positive correlation between depression severity and normalized brain activity was found in the right amygdala ($r = 0.73$, $p = 0.005$) and in the right anterior cingulate ($r = 0.55$, $p = 0.019$). A negative correlation was found in the left dorsal lateral prefrontal cortex ($r = -0.60$, $p = 0.008$).

Discussions: Neural activity and depression showed significant covariation both in direction and location within brain structures known to participate in the mechanisms of depression without gross brain damage. These results are consistent with an overlap between the systems neuroscience mechanisms of *functional* depression and depression following left basal ganglia stroke.

Disclosure: S. Paradiso: None. K. Ostedgaard: None. J. Vaidya: None. R. Robinson: None.

227. Default Mode Network Abnormalities in Insulin Resistant Postmenopausal Women Receiving Estrogen Therapy

Natalie Rasgon*

Stanford University, Stanford, CA

Background: Increasing data from functional imaging studies suggest the importance of the default mode network (DMN) in identifying abnormal functioning in the aging brain. Alterations in DMN activity have been observed in healthy older adults with established dementia risk factors (i.e. APOE4). The present study sought to investigate DMN activity with respect to insulin resistance (IR), which is also thought to increase risk for dementia and cognitive decline in the aging brain.

Methods: Subjects were 20 postmenopausal women receiving estrogen therapy for at least one year; half of the women had biomarkers of IR as characterized by elevated fasting plasma insulin level and body mass index, while the other half had biomarkers suggesting insulin sensitivity (IS) as characterized by low levels of fasting plasma insulin and normal body mass index. All subjects underwent resting state functional magnetic resonance imaging (fMRI) and testing of executive function. Correlations and anti-correlations between DMN brain regions and the hippocampus were examined within each group and between groups. Executive function was also compared between the two groups.

Results: One-sample t-tests in each group (IR and IS) showed reciprocal positive correlations between the left and right hippocampus, as well as reciprocal positive correlations amongst the main DMN nodes, i.e. the medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC), and left and right lateral parietal cortices (LlLP and RtLP, respectively). Additionally, the IS group showed significant positive correlation between the MPFC and bilateral hippocampus.

No anticorrelations were observed amongst these regions. Two-sample t-tests showed that IR subjects had significantly less correlation between the MPFC and bilateral hippocampus relative to the IS subjects. IR subjects also demonstrated significantly worse executive function compared to IS subjects.

Discussion: We report significantly less DMN-hippocampal connectivity and significantly worse executive function among IR women compared to IS women. Further investigation on the potential utility of DMN activity and IR as concurrent biomarkers of pathological aging is warranted.

Disclosure: N. Rasgon: Part 1; Bayer HealthCare Inc., Pfizer, Wyeth-Ayerst, Bristol-Myers Squibb, Abbott Laboratories, GlaxoSmithKline.

228. Amygdala to Nucleus Accumbens Excitatory Transmission Facilitates Reward Seeking

Garret Stuber*, Dennis Sparta, Wieke Van Leeuwen, Juanita Hardjoprajitno, Saemi Cho, Kay Tye, Kimberly Kempadoo, Feng Zhang, Karl Deisseroth, Antonello Bonci

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

Background: The basolateral amygdala (BLA) plays a crucial role in emotional learning irrespective of valence. While the BLA projection to the nucleus accumbens (NAc) is hypothesized to modulate cue-triggered motivated behaviors our understanding of the interaction between these two brain regions has been limited by the inability to selectively manipulate neural circuit elements of this pathway during behavior.

Methods: To circumvent this limitation, we used *in vivo* optogenetic stimulation of glutamatergic fibers from the BLA to the NAc, coupled with intra-cranial pharmacology and *in vitro* electrophysiology. Adult male mice were injected into the BLA with adeno-associated virus coding for the light-sensitive cation channel, channelrhodopsin-2 (ChR2) expressed under control of the CaMKIIα promoter to predominantly target glutamatergic neurons. Following 3–4 weeks after surgery, mice were either prepared for patch-clamp electrophysiological experiments to selectively study BLA-to-NAc excitatory neurotransmission, or used in behavioral experiment to test whether pathway-specific stimulation of BLA-to-NAc excitatory fibers could promote reward seeking.

Results: We found that optical stimulation of the BLA-to-NAc pathway resulted in robust excitatory post-synaptic currents in brain slices in mice reinforces behavioral responding to earn additional optical stimulations of these synaptic inputs. The behavioral responding observed was dependent on receiving optical stimulation of the pathway as mice readily extinguished responding when laser stimulations were withheld. Furthermore, optical stimulation of BLA-to-NAc glutamatergic fibers required intra-NAc dopamine D₁-type, but not D₂-type receptor signaling as microinjection of the D₁-specific antagonist, SCH23390, into the NAc significantly reduced optical self-stimulation behavior. Moreover, while optical stimulation of medial prefrontal cortex (mPFC) to NAc glutamatergic fibers also elicited reliable excitatory synaptic responses, optical self-stimulation behavior was not observed by activation of this specific pathway.

Discussion: These data suggest that while the BLA is important for processing both positive and negative affect, the BLA-to-NAc glutamatergic pathway in conjunction with dopamine signaling in the NAc promotes responding for stimuli of positive valence.

Disclosure: G. Stuber: None. D. Sparta: None. W. van Leeuwen: None. J. Hardjoprajitno: None. S. Cho: None. K. Tye: None. K. Kempadoo: None. F. Zhang: None. K. Deisseroth: None. A. Bonci: None.

229. Amphetamine-Induced Locomotor Conditioning Requires CDK5 in the Nucleus Accumbens: Implications for Understanding Changes in Dendritic Spine Morphology Following Stimulant Exposure

Paul Vezina*, Bryan F Singer

The University of Chicago, Chicago, IL

Background: The induction of drug-induced sensitization necessarily involves exposure to the drug in association with a complex of environmental stimuli. Similarly to what has been shown to occur following repeated intermittent exposure to systemic amphetamine, exposure to amphetamine in the ventral tegmental area (VTA) sensitizes drug-induced locomotion and nucleus accumbens (NAcc) dopamine overflow and enhances drug self-administration. However, unlike what occurs following systemic amphetamine, exposure to VTA amphetamine does not produce conditioning and fails to increase dendritic spine density, dendritic length, or dendritic branching in the NAcc. These results suggest that psychostimulant-induced increases in dendritic morphological traits may correspond to associative conditioning rather than non-associative sensitization [Singer *et. al.* (2009) *Biol Psychiatry* 65, 835–40]. To further evaluate this possibility, the present experiment aimed to inhibit conditioning, while preserving sensitization, by blocking the increases in NAcc dendritic spine density normally observed in rats exposed to systemic amphetamine.

Methods: This was achieved by infusing the cyclin-dependent kinase 5 (cdk5) inhibitor (R)-Roscovitine into the NAcc of rats during exposure to amphetamine. NAcc (R)-Roscovitine has been shown to block increases in cocaine-induced dendritic spine density while preserving behavioral sensitization in rats [Bibb *et. al.* (2001) *Nature* 410, 376–80; Norrholm *et. al.* (2003) *Neuroscience* 116, 19–22; Taylor *et. al.* (2007) *PNAS* 104, 4147–52]. In the present experiments, drug-exposure consisted of four 3-day conditioning blocks. Injections were given on the first two days of each block (the first in the open field and the second in the home cage), followed by a procedure-free day. Rats received microinjections of saline or (R)-roscoxitine (40 nmol/0.5 μl/ side) into the NAcc 30 minutes before being administered a systemic amphetamine (1.0 mg/kg, IP) or saline injection. Rats in the Paired group received NAcc saline or roscovitine followed by IP amphetamine in the open field and systemic saline in the home cage. Rats in the Unpaired group received systemic saline in the open field and NAcc saline or roscovitine followed by IP amphetamine in the home cage. Rats in the Control group received IP saline in both environments preceded by either NAcc roscovitine or NAcc saline microinjections. After a one week procedure free period, all rats were administered an IP saline challenge injection in the open field and their locomotor activity assessed.

Results: NAcc cdk5 blockade during the amphetamine-environment pairings prevented the development of conditioned locomotion. Conditioned locomotion on the test was observed in Paired rats relative to rats in the Unpaired and Control groups but it was reduced in the Paired rats administered the NAcc roscovitine microinjections in the conditioning phase.

Discussion: Since NAcc cdk5 inhibition prevents drug-induced increases in dendritic spine density, these results are consistent with our previous findings indicating that this change in dendritic morphology correlates with associative conditioning rather than non-associative sensitization.

Disclosure: P. Vezina: None. B. Singer: None.