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ACNP 50th Annual Meeting
Poster Abstracts

Poster Session I
Monday, December 5, 2011 5:30 PM – 7:30 PM

1. Psychostimulant Treatment of Age-related Cognitive Decline: Attention Enhancing Effects of Methylphenidate are Reduced in Senescent Rats

Barry D. Waterhouse*, Richard Chu, Jed S. Shumsky, Shevon E. Nicholson

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Background: Background: Methylphenidate (MPH) - Ritalin®, a psychostimulant that blocks reuptake of synaptically released norepinephrine and dopamine in the brain, is used clinically to treat a variety of disorders where cognitive function is compromised. The drug is also gaining popularity among healthy individuals for its ability to promote wakefulness, focus attention, and improve concentration. Elderly individuals may be particularly prone to use MPH, but the efficacy of the drug for cognitive enhancement in senescent individuals has not been demonstrated. Studies in our laboratory have focused on the actions of MPH in normal, adult male Sprague Dawley rats (3-6 months old) and have determined that low dose MPH (6.0-8.0 mg/kg oral) enhances performance in a visually-guided sustained attention task (modified from McGaughy and Sarter, 1995) and in an attention set-shifting task (modified from Birrell and Brown, 2000). We hypothesized that low dose MPH would also improve vigilance task and set-shifting task performance in aged animals.
Methods: Methods: Adult (3-6 months) and aged (18-22 months) male Fischer 344 rats were given MPH (2.0-12.0 mg/kg oral), and their dose-dependent performance was tested in both the sustained attention task and a locomotor activity chamber. Adult and aged rats were also given 8.0 mg/kg oral MPH and tested in the attention set-shifting task.
Results: Results: The results indicate that oral MPH (6.0-8.0 mg/kg) improves vigilance and set shifting task performance in adult Fischer 344 males; but there is no improvement in task performance in the aged animals from this strain. Aged Fischer 344 rats also demonstrated increased motor side effects as a result of MPH administration (8.0-12.0 mg/kg). Similar results have been obtained in preliminary studies of MPH action and attention set shifting in adult vs. aged male Sprague-Dawley rats.
Discussion: Discussion: Taken together these outcomes suggest that use of MPH for cognitive enhancement in elderly individuals may be both ineffective and likely to produce untoward motor side effects. Future investigations will explore the basis for MPH's lack of efficacy in the aging brain, but an initial postulate focuses on age-related loss of catecholamine neurons and reduced forebrain projections from locus coeruleus and ventral tegmental areas in senescent animals. Support: NIH NIDA DA017960, NIMH MH087921.
Disclosure: B. Waterhouse: None. **R. Chu:** None. **J. Shumsky:** None. **S. Nicholson:** None.

2. Using Translatable Human Biomarkers to Assess Clinical Relevance of Mouse Models of Obsessive Compulsive Disorder

Susanne E. Ahmari*, Victoria B. Risbrough, Lauren Leotti, Cara Malapani, Edward E. Smith, Mark A. Geyer, Rene Hen, H. Blair Simpson

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Background: Though Obsessive Compulsive Disorder (OCD) is one of the most disabling and chronic psychiatric disorders, with 2-3% lifetime prevalence, the pathophysiology remains unclear. This is partly because it is difficult to make OCD mouse models that recapitulate symptoms in multiple cognitive, behavioral, and emotional domains: obsessive thoughts; compulsive behaviors; and anxiety. To develop new OCD mouse models that may recapitulate more than one symptom domain, we are using an optogenetic approach to modulate activity in specific OCD-relevant brain circuits. To validate the clinical relevance of these mouse models, we are performing parallel studies in OCD patients to identify translatable biomarkers that correlate with OCD symptoms.
Methods: Humans: 22 unmedicated OCD subjects and matched healthy controls were tested in prepulse inhibition (PPI) and Stop Signal Reaction Time (SSRT) task. Clinical measures were obtained at time of testing (YBOCS, OCI-R, HAM-D, YGTSS, STA-I, ASI). Mice: Optogenetic techniques were used to stimulate corticostriatal pathways; anxiety and repetitive behaviors were measured with open field and elevated plus maze paradigms.
Results: Humans: Repeated measures ANOVA indicated that PPI was impaired in unmedicated OCD subjects ($p<.015$). OCD subjects with a history of tics ($n=3$) had significantly lower percent PPI than OCD patients without a history of tics ($p<.045$). OCD subjects' ability to inhibit behavioral responses had a non-statistically significant improvement compared to matched controls (i.e. decreased SSRT; $p<.08$), accompanied by an overall slower reaction time in OCD patients and increased accuracy.
Mice: We have developed optogenetic technology that enables selective stimulation of cortico-striatal-thalamic circuits implicated in OCD. Preliminary results indicate that selective stimulation of projections from orbitofrontal cortex (OFC) to striatum leads to repetitive behaviors, but does not change anxiety levels.
Discussion: We have demonstrated abnormalities in two translatable neurocognitive tasks (PPI, SSRT) in unmedicated OCD patients vs matched healthy controls. 1) Our human results confirm that PPI abnormalities are seen in OCD subjects, and support use of PPI to help validate OCD mouse models. 2) Though we also see abnormalities in the SSRT, our findings run counter to the published literature (i.e. we see improved inhibition in OCD subjects); this result can be reconciled by our finding that OCD patients sacrificed speed on the task in favor of improving overall accuracy (i.e. the speed-accuracy tradeoff). This response profile suggests caution in using this task as a reliable translatable biomarker. 3) Preliminary optogenetic results in mice demonstrate that selective stimulation of OFC-striatal circuits may recapitulate

some OCD-relevant behaviors (repetitive movements), but not others (anxiety). 4) Identification of additional reliable translatable biomarkers will increase our ability to validate mouse models that can be used to dissect molecular substrates underlying OCD pathophysiology.
Disclosure: S. Ahmari: None. **V. Risbrough:** Part 1: Arena, Cenomed, Ferring Pharmaceuticals. **L. Leotti:** None. **C. Malapani:** None. **E. Smith:** None. **M. Geyer:** Part 1: Acadia, Abbott, Addex, Cerca Insights, Merck, Omeros, Sepracor, Takeda, and Teva. Equity interest in San Diego Instruments, Inc., Part 2: Omeros - consulting San Diego Instruments - Equity interest, Part 4: Johnson & Johnson, IntraCellular Therapeutics. **R. Hen:** Part 1: Dr. René Hen receives compensation as a consultant for BrainCells, Inc., PsychoGenics Inc., Memory Pharmaceuticals, Roche, Astra Zeneca, and Lundbeck., Part 4: Astra Zeneca. **H. Simpson:** Part 1: Janssen Pharmaceuticals, Pfizer Inc., Neuropharm Ltd, Transcept Pharmaceuticals, Part 4: Neuropharm Ltd, Transcept Pharmaceuticals.

3. Repeated Psychological Trauma Causes Enduring Corticotropin-Releasing Factor-Dependent Sensitization of Basolateral Amygdala Noradrenergic Systems: A Substrate for PTSD-like Startle and Sensorimotor Gating Abnormalities?

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Background: Prepulse inhibition (PPI), a measure of preattentional sensorimotor gating mechanisms that are deficient in numerous psychiatric illnesses, refers to the diminution of startle responses when weak prestimuli immediately precede the startling stimulus. PPI is a reliable and reproducible paradigm with which to measure in humans and model in animals the information-filtering deficits seen in schizophrenia and posttraumatic stress disorder (PTSD), and as such has the potential to elucidate basic neurobiological mechanisms that may contribute to these illnesses. Stress is involved in schizophrenia, in which symptom relapse can occur following major life stressors, and PTSD, which is triggered by intense stress that is perceived of as potentially life-threatening. Yet surprisingly, stress-induced effects on PPI are not well characterized, particularly using animal models that capture the unique sequelae of psychological trauma. Such information could be very important in understanding the neural substrates involved in the exacerbation of these illnesses by stress. Two systems involved in stress are corticotropin-releasing-factor (CRF) and norepinephrine (NE), each of which is implicated in PPI and is highly expressed within stress-responsive sites like the amygdala. Whether these systems interact or participate in stress-induced PPI effects via this site has not been explored.
Methods: Using predator stress (rat in protective cage within ferret's homecage for 5 min), we determined if repeated stress altered the functioning of amygdala CRF and NE systems in a way that would promote deficient PPI. First, separate groups of male Sprague Dawley rats received CRF (200 ng), NE (20 μg), or vehicle into basolateral (BLA) or central amygdala (CeA) on 3 test days; vehicle- and CRF-treated rats were challenged subsequently with a subthreshold dose of NE (0.3 μg) and NE-treated rats were challenged with CRF. PPI was measured after each infusion. Another group underwent the same regimen, but instead of infusions got 3 exposures to predator stress and was challenged with intra-BLA NE (0.3 μg) or a subthreshold dose of yohimbine. Additional groups underwent the same repeated ferret protocol, but received intra-BLA infusion of the CRF1 receptor antagonist NBI27914 (1 μg) either just before or 30 min after each ferret exposure, or just before the intra-BLA NE challenge. Finally, additional groups were exposed to the repeated ferret regimen (without drugs) and then challenged with 30 μg of either the α1 NE agonist phenylephrine or the β agonist isoproterenol in the BLA.
Results: Intra-BLA CRF had no effect acutely, however repeated infusions caused a profound PPI deficit ($P < 0.05$) in response to subsequent intra-BLA infusion of a low, subthreshold dose of NE. This NE sensitization was also seen in rats exposed to predator stress ($P < 0.01$). Repeated predator stress also enhanced startle reactivity to low-dose intra-BLA NE ($P < 0.01$), but this effect emerged later than the sensitization to PPI deficits. NE system hypersensitivity was evident even 28d after stress termination when a subthreshold dose of systemic yohimbine disrupted PPI in predator-exposed rats ($P < 0.05$). This phenomenon was specific to BLA, as intra-CeA infusions had no effect, and was unique to CRF-receptor stimulation, since repeated BLA NE produced neither homotypic nor heterotypic sensitization. Intra-BLA NBI27914 completely blocked predator-stress-induced effects when the CRF1 antagonist was given prior to stress, but not when given 30 min post-stress or immediately before the intra-BLA NE challenge. Finally, simultaneous stimulation of α1 and β receptors is necessary to disrupt PPI via BLA in non-stress-exposed rats, but after repeated predator stress, stimulation of either receptor on its own was sufficient ($P < 0.01$).
Discussion: Repeated stress exposure using a rodent analog of what may be experienced as potentially life-threatening psychological trauma leads to long-lasting plasticity of BLA systems such that subthreshold NE levels in this site are now capable of disrupting PPI and elevating startle long after the stress has ended. BLA CRF1 receptors are necessary for the development but not the expression of this enduring stress-induced neuroadaptation, which may involve a functional 'uncoupling' of α1 and β NE receptors that promotes enhanced sensitivity to low-level stimulation. Given the major role of intense emotional stress in schizophrenia and PTSD symptoms, these findings could indicate a possible neural substrate underlying the deficient sensorimotor gating and stimulus hyperreactivity associated with these illnesses.
Disclosure: V. Bakshi: None. **A. Rajbhandari:** None.

4. Rapid Tryptophan Depletion Moja-De decreases Serotonergic Function and influences Anxiety-Like Behavior in a Strain-Selective Way in Mice

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Background: Rapid tryptophan depletion (RTD) is a method of decreasing plasma levels of tryptophan (TRP) and is widely used in neuropsychiatric and pharmacological research to temporarily suppress serotonergic function in the human brain. Common side-effects of RTD are vomiting and nausea, but a new RTD protocol (RTD Moja-De) with a body weight-adapted administration and modified amino acid (AA) composition has proven to have better tolerance. The purpose of the present investigation was to determine if RTD, using this improved mixture, would suppress central nervous serotonin (5-HT) function in vulnerable but not resistant mouse strains. We tested Moja-De in C57 BL/6J (C57) mice, which have been reported to be resistant to the effects of RTD and BALB/cJ mice (BALBc) which have a mutation in TPH2 which lowers their baseline 5-HT synthesis and would be predicted to exhibit an exaggerated response to RTD. We hypothesized that Moja-De would significantly lower brain 5-HT synthesis and serotonergic activity, without affecting dopamine or norepinephrine.
Methods: Mice were treated with two doses of 2 g AAs/kg BW mixed with 10 ml/kg BW deionized water spaced 30 minutes apart. Male BALB/cJ mice and male C57 BL/6 mice from Jackson

Laboratory (Bar Harbor, Maine, USA) were used. Anxiety-like behavior was measured using the Light/Dark-Test 2 hours after the second treatment with a TRP + (= control) mixture, TRP- (= RTD) or water. For neurochemical data animals were decapitated 2 hours after the second treatment; prefrontal cortex, frontal cortex and hippocampus were collected. Brain neurotransmitter and metabolite content were quantified using a reverse phase high-performance liquid chromatography (RP-HPLC) system with electrochemical detection. Each dependent measure (TRP, 5-HTP, 5-HT, 5-HIAA, NE, DA) was analyzed for all groups (water, TRP-, TRP +) using a global 4-way ANOVA repeated measures analysis of variance with follow-up lower order ANOVAs and post-hoc Newman-Keuls-Test to identify differences between specific treatment groups.
Results: TRP + and TRP- diets effectively raised and lowered TRP in the brain respectively. The effects of the TRP manipulations were similar in BALBc and C57 strains.TRP- decreased 5-HT synthesis in all brain regions, but effects on C57 were greater than in BALBc mice. At baseline, BALBc mice had a lower level of 5-HT synthesis, lower 5 HIAA and lower 5-HT content in the hippocampus and the prefrontal cortex than C57.TRP + did not increase 5-HT synthesis or 5-HT content. TRP- significantly decreased 5-HT content; TRP- was consistently lower than TRP + in all regions. 5-HIAA was decreased by TRP- in every brain region investigated. DA, DOPAC and HVA did not show any strain differences or treatment effects. Norepinephrine was significantly lower in BALBc than C57. Treatment did not affect norepinephrine levels.There were no strain differences in baseline behaviors in the water treated animals. However RTD had strain-specific effects: TRP- treated BALBc mice spend less time in the light and covered less distance in the light compared to TRP + BALBc mice. In C57 s the opposite effect was observed: TRP- treated mice spent more time in the light and covered more distance in the light than TRP + animals.
Discussion: This study demonstrated that the treatment with RTD Moja-De (TRP-) decreased brain TRP and subsequently 5-HTP, 5-HT and 5-HIAA in both strains of mice, but was more effective in C57 mice than in BALBc mice. One possible explanation is that the decreased levels of TPH2 in BALB/c are offset by the increased affinity for TRP exhibited by this mutation which might render it less sensitive to physiologic variation in TRP availability.The balanced AA mixture (TRP +) increased TRP levels, but did not enhance 5-HT synthesis. Despite the unexpected neurochemical effects, TRP- did cause increased anxiety-like behavior in the strain we predicted would be vulnerable to RTD. RTD Moja-De caused an anxiogenic response in BALBc mice whereas C57 showed an anxiolytic effect. Studies show that the characterization of BALBc depends upon whether social or nonsocial tasks are used. The opposite effect of RTD on behavior suggests that differences in serotonergic function contribute to the behavioral differences observed in BALBc mice. We found that BALBc mice have significantly lower norepinephrine levels in all brain regions compared to C57 mice. This could be a supplementary explanation for the anxious phenotype of BALBc mice.
Disclosure: C. Biskup: None. **A. Arrant:** None. **A. Day:** None. **C. Kuhn:** None. **F. Zepf:** Part 4: FDZ was the recipient of an unrestricted award donated by the American Psychiatric Association (APA), the American Psychiatric Institute for Research and Education (APIRE), and Astra Zeneca ("Young Minds in Psychiatry Award"). He has also received research support from the German Federal Ministry of Economics and Technology (Bundesministerium für Wirtschaft und Technologie, BMWi), the German Society for Social Pediatrics and Adolescent Medicine (Deutsche Gesellschaft für Sozialpädiatrie und Jugendmedizin, DGSPJ), and from the Paul and Ursula Klein Foundation, which is unrelated to the present investigation. He was the recipient of a travel stipend donated by the GlaxoSmithKline Foundation and an unrestricted educational grant donated by Shire pharmaceuticals.

5. MicroRNA Regulation of Fear Extinction Memory
Timothy W. Bredy*

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Background: Extinction, the gradual reduction in conditioned fear responses generated by repeated presentation of a non-reinforced conditioned stimulus, is a process of inhibitory learning with enormous clinical importance, since it represents the explicit model of behavioural therapy for phobia, panic, and post-traumatic stress disorder. Understanding the neural mechanisms by which fear can be extinguished is crucial if we are to develop better therapeutic protocols for the treatment of affective disorders. MicroRNAs (miRs) are a newly discovered family of endogenous small non-coding RNAs ($\sim$23 nucleotides long) that regulate gene function either by degrading target mRNAs or by direct complimentary binding to the 3′UTR of protein-coding genes, resulting in post-transcriptional silencing. In the past decade, several lines of evidence have implicated various miRs in the pathogenesis of human brain disorders, including schizophrenia; however, their functional role in fear-related learning and memory remains largely unknown.
Methods: We used a combined approach of *in vitro* assays and *in vivo* lenti-viral mediated gene transfer to elucidate the role of miR-128b in the formation of fear extinction memory.
Results: We've discovered that the brain-specific microRNA, miR-128b, is highly expressed within excitatory cortical neurons that express its host gene, regulator of calcium signaling (RCS), and that are innervated by dopamine within the infralimbic prefrontal cortex (ILPFC); a brain region heavily involved in encoding fear extinction memories. We also show that, unlike the non-specific expression pattern of brain-specific miR-134, miR-128b is preferentially increased in the ILPFC after extinction but not after the acquisition of conditioned fear. Using an *in vivo* lentiviral-mediated gene transfer approach and *in vitro* luciferase assays, we have found a functional relationship between miR-128b and several predicted target genes known to be involved in learning-related synaptic plasticity.
Discussion: Our data suggest an important role for miR-128b in regulating long-term memories associated with fear extinction.
Disclosure: T. Bredy: None.

6. Antagonism of Kappa-Opioid Receptors reduces Stress-Induced Effects
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Background: Brain kappa-opioid receptors (KORs) are implicated in stress-related behaviors. Previously we have shown that systemic KOR antagonism produces antidepressant- and anxiolytic-like effects in a variety of tests including fear-potentiated startle and the elevated plus maze. Recently it has been shown that KOR antagonism can also block behavioral and neurochemical effects of corticotropin-releasing factor (CRF), the principal regulator of the stress response. The present studies were designed to further characterize interactions between KOR systems and stress-induced behavior using methods of anxiety-like behavior that rely on potentiation of the acoustic startle reflex as a dependent measure. Footshock and central administration of CRF are two methods that can be used to potentiate the acoustic startle reflex.
Methods: To test the effect of KOR antagonism on shock-potentiated startle, mice were matched into groups with equivalent baseline startle and given an intraperitoneal (IP) injection of the KOR antagonist JDTic (10 or 30 mg/kg) or vehicle 24 hr prior to testingto accommodate the slow onset and persistent actions of JDTic. In the test session, mice received a baseline startle test

followed by ascending footshock amplitudes (0.2 mA, 0.4 mA, and 0.8 mA) each followed by a startle test. The following day, shocked mice were given a final startle test. In CRF-enhanced startle, mice were pretreated 7 days before testing with JDTic (10 or 30 mg/kg, IP). Mice were then matched into groups with equivalent baseline startle. Immediately prior to the test session, mice received an intracerebroventricular (ICV) infusion of CRF (1 μg) or vehicle and were placed into the startle chamber. Acoustic startle to a white noise burst was measured throughout a 200 min session. To identify neuronal activation in brain areas involved in anxiety, c-Fos expression was examined. Mice were pretreated with JDTic (30 mg/kg, IP), then given an infusion of CRF (1 μg, ICV) or vehicle exactly as in the behavioral tests. Two hours later they were perfused for c-Fos immunohistochemistry. Data were analyzed using appropriate ANOVAs and significant effects were further analyzed using *post hoc* Bonferonni tests.

Results: Blockade of KOR receptors by JDTic at 30 mg/kg attenuated footshock induced increases in startle amplitude, though not significantly. However, when the shocked mice were returned to the testing chamber on the following day, JDTic pretreatment significantly decreased context conditioning. CRF produced a marked increase in startle amplitude; this effect was dose-dependently attenuated by JDTic, with nominal reductions at 10 mg/kg and significant reductions at 30 mg/kg. C-Fos immunohistochemistry was then used to determine brain regions that may be involved in these effects. Consistent with the literature, CRF was found to activate numerous brain regions including the central and basolateral nuclei of the amygdala, bed nucleus of the stria terminalis, piriform cortex, ventral tegmental area (VTA), and dentate gyrus (DG) of the hippocampus. KOR antagonism with JDTic significantly reduced CRF-induced increases in c-Fos labeling in the VTA and DG.

Discussion: These studies provide additional evidence that blockade of KORs reduce anxiety-like behavior in mice. Although JDTic did not significantly decrease shock-potentiated startle, startle amplitude in the shock context the following day was significantly reduced. This suggests JDTic may protect against detrimental neuroadaptations that occur following a stressful event. KOR antagonism also reduced the effects of CRF on startle, supporting the existence of functional interactions between KOR systems and CRF. The decreases in c-Fos labeled neurons in the VTA and the DG suggest the hippocampus and midbrain dopamine cells play important roles in mediating these effects. These data suggest blockade of KORs may be a useful tool in the treatment and prevention of stress-induced illness.

Disclosure: A. Van't Veer: None. **F. Carroll:** None. **W. Carlezon:** Part 1: Scientific Advisory Board, Myneurolab.com Consultant, Concert Pharmaceuticals.

7. Epigenetic Differences in the Developing Hippocampus and Amygdala in a Novel Rat Model of Anxiety and Depression

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Background: Innate differences in human temperament strongly influence how individuals cope with stress and predispose for specific types of psychopathology. The present study examines developing brain circuits in an animal model of temperamental differences to understand how altered neurodevelopment may engender differences in emotional reactivity that are stable throughout the animal's life, and may put an individual at risk for a depressive/anxious phenotype.

Methods: We utilize selectively-bred High Responder (bHR) and Low Responder (bLR) rats that exhibit dramatic emotional behavior differences, with bHRs exhibiting exaggerated novelty-exploration, aggression, impulsivity and drug self-administration, and bLRs showing marked behavioral inhibition, exaggerated anxiety- and depressive-like behavior. Using Affymetrix microarrays, we assessed bLR/bHR gene expression in the developing brain on postnatal days (P)7, 14, and 21, focusing on the hippocampus and nucleus accumbens, two regions related to emotionality and known to differ in adult bLR/bHR rats. Stereological studies were used to examine potential differences in brain structure.

Subsequent studies began to examine potential epigenetic mechanisms (e.g. DNA methylation) that may contribute to putative gene expression differences in the developing bHR versus bLR brain. To this end, we used *in situ* hybridization to assess the mRNA expression of DNA methyltransferase 1 (DNMT1) – one of the chief enzymes involved in DNA methylation in the brain.

Results: The microarray study revealed dramatic bLR/bHR gene expression differences in the P7 and P14 hippocampus, with minimal differences in the nucleus accumbens. Some of the most profound differences involved genes critical for neurodevelopment and synaptogenesis. Stereological studies evaluated hippocampal structure in developing bHR/bLR pups, revealing enhanced hippocampal volume and cell proliferation in bLR animals. Results of the DNMT1 *in situ* hybridization study point to potential bHR/bLR differences in DNA methylation within specific subregions of the hippocampus (upper blade of the dentate gyrus and the CA3 region). Interestingly, additional analysis also uncovered similar bHR/bLR DNMT1 differences within select nuclei of the amygdala – another key brain region that controls emotional behavior, in part via reciprocal connections with the hippocampus.

Discussion: Our work-to-date with the bHR/bLR rat lines demonstrates the heritability of the bHR and bLR behavioral phenotypes. Their underlying genetic differences appear to drive distinct formation of the hippocampus, leading to marked differences in hippocampal morphology and gene expression during the first two weeks of life. The DNMT1 findings in the hippocampus and amygdala suggest that the observed bHR/bLR differences in gene expression, brain morphology and behavior may be linked to epigenetic changes occurring in early life. Ongoing efforts will continue to examine DNA methylation status in the developing bHR/bLR hippocampus and select amygdalar nuclei, and determine whether early-life interventions (such as environmental enrichment) known to improve LRs' anxious/depressive phenotype act by influencing hippocampal DNA methylation levels. Taken together, this body of work may provide important insight into the possible genesis of individual differences in emotionalty and related risks for the emergence of emotional disorders (e.g. the anxiety-prone nature of bLRs or drug addiction proclivity of bHRs).

Disclosure: S. Clinton: None. **R. Simmons:** None. **D. Simpson:** None. **S. Watson:** None. **H. Akil:** None.

8. Fear Conditioning in Rat Pups and Fear Reactions Acquired from Exposure to Fearful Mothers involve Similar Brain Structures

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Background: Existing research demonstrates that emotional trauma may be transmitted across generations. For example, Holocaust studies suggest that adult children of Holocaust survivors diagnosed with posttraumatic stress disorder (PTSD), similarly to their parents, experience psychological distress associated with Holocaust-related cues (Yehuda *et al.*, 1998). Animal studies so far investigating the impact of parental stress on

the offspring focused on the overall behavioral alterations in the offspring. We have recently demonstrated that exposure of rat pups to mothers fearful in response to previously conditioned olfactory cue produces fear responses in pups that are maternal-conditioned-cue-specific (Debiec and Sullivan; SFN 2010). Here we investigate the neural basis of the mother-to-infant transfer of learned fear.
Methods: Rat pups were exposed to fearful mothers ('Paired/Re-exposed' - mother that had previously received olfactory fear conditioning, now re-exposed with pups to the conditioned cue). Controlled groups included pups exposed to mothers that had been previously conditioned but were not re-exposed to the conditioned cue ('Paired/Not Re-exposed') and pups exposed to mothers that had been previously exposed to unpaired presentations of odor and electric shock ('Unpaired/Re-exposed'). The number of pups in each group was equal (4 pups). All pups were injected with ^{14}C 2-DG prior to their exposure in order to assess the neural changes during acquisition. Following exposure to fearful (non-fearful) mothers, 2-DG reuptake in pups' brains was assessed. In another set of experiments, a separate group of rat pups underwent olfactory fear conditioning ('Paired' group; $n = 6$), unpaired olfactory cue and electric footshock presentations ('Unpaired' group; $n = 6$), and cue only presentations ('Cue only'; $n = 4$). Re-uptake of 2-DG in all groups was assessed.
Results: In mother-to-infant transfer of fear experiments, analysis of variance (ANOVA) revealed that infants from the 'Paired/Re-exposed' group exhibited increased mean 2-DG uptake in the dorsal part of lateral nucleus of the amygdala (LA) [ANOVA, $F(2,9) = 6.035$; $p < 0.05$]. *Post hoc* Fisher's tests indicated that pups from 'Paired/Re-exposed' group significantly differed from each of the other groups at the $p < 0.05$ level. In pup fear conditioning experiments, ANOVA revealed that pups from the 'Paired' group exhibited increased mean 2-DG uptake in the dorsal part of LA [ANOVA, $F(2,10) = 5.863$; $p < 0.05$]. *Post hoc* Fisher's tests indicated that pups from 'Paired' group significantly differed from each of the other groups at the $p < 0.05$ level.
Discussion: Our data demonstrate that acquisition of conditioned fear in rat pups in involves the LA, a key structure in fear conditioning in adult rats (LeDoux, 2000). Interestingly, our data suggest that the LA in rat pups is also involved in acquiring cue-specific fear responses from fearful mother. Mother-to-infant transfer of fear experience may involve similar brain structures and mechanisms that are implied in fear conditioning. Further studies of neurobehavioral basis of transgenerationally transmitted fear experience will allow a development of early therapeutic interventions.
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Disclosure: J. Debiec: None. **R. Sullivan:** None.

9. Essential Role for Orbitofrontal 5-HT1B Receptors in OCD-Like Behavior and SRI Response in Mice

Nancy A. Shanahan, Lady P. Velez, Virginia L. Masten, Stephanie C. Dulawa*

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Background: Perseveration and sensorimotor gating deficits are core features of obsessive compulsive disorder (OCD). Serotonin 1B receptor (5-HT1BR) agonists exacerbate OCD symptoms in patients, and induce perseveration and sensorimotor gating deficits in mice. Serotonin reuptake inhibitors (SRIs), but not noradrenaline reuptake inhibitors (NRIs), reduce OCD symptoms following 4–8 weeks of treatment. Furthermore, 4 weeks of treatment with SRIs, but not NRIs, block 5-HT1BR agonist-induced perseveration and sensorimotor gating deficits in mice. We compared the effects of chronic SRI versus NRI treatment on 5-HT1BR expression and Gprotein-coupling in orbitofrontal-subcortical "OCD circuits", and localized the 5-HT1BR population that mediates OCD-like behavior.
Methods: Mice chronically received the SRIs clomipramine or fluoxetine, or the NRI desipramine and were examined for 5-HT1BR binding and G-protein-coupling in the caudate-putamen, nucleus accumbens, orbitofrontal cortex, and dorsofrontal cortex. Finally, OCD-like behavior was assessed following intra-orbitofrontal 5-HT1BR agonist infusion, or intra-orbitofrontal 5-HT1BR antagonist infusion coupled with systemic 5-HT1BR agonist treatment.
Results: Effective, but not ineffective, OCD treatments down-regulated 5-HT1BR expression specifically in the orbitofrontal cortex. Intra-orbitofrontal 5-HT1BR agonist infusion induced OCD-like behavior, and intra-orbitofrontal 5-HT1BR antagonist infusion blocked the OCD-like effects of systemic 5-HT1BR agonist treatment.
Discussion: Our findings indicate that orbitofrontal 5-HT1BRs are necessary and sufficient to induce OCD-like behavior in mice, and that SRI pharmacotherapy reduces OCD-like behavior by desensitizing orbitofrontal 5-HT1BRs. Our findings suggest an essential role for orbitofrontal 5-HT1BRs in OCD pathophysiology and treatment.
Disclosure: N. Shanahan: None. **L. Velez:** None. **V. Masten:** None. **S. Dulawa:** None.

10. Exposure to Traumatic Stress in Rats Differentially Affects Alcohol-Related Behaviors and Brain ERK Phosphorylation

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Background: PTSD and alcoholism are two disorders that exhibit a high degree of co-morbidity in humans and have partially overlapping symptomatic profiles. The aim of this study was to examine the effects of traumatic stress on alcohol-related behaviors.
Methods: Male Wistar rats were trained to respond for 10% w/v ethanol vs. water on a continuous reinforcement schedule in a two-lever operant situation. Rats were then exposed to two contexts that differed on two sensory modalities (visual & tactile stimuli). Rats were divided into two groups (conditioned & controls) matched for chamber preference and ethanol responding. Conditioned rats were exposed to neutral odor in non-preferred context and predator odor (bobcat urine) in preferred context, then tested 24 hrs later for conditioned place avoidance (CPA). Conditioned rats were divided into two groups (avoiders & non-avoiders) based on avoidance of predator odor-paired context relative to baseline (avoiders defined by $\geqslant$ 10-s decrease in time spent in predator odor-paired context during 5-min test). Rats were tested for operant ethanol responding on days 2, 5, 8 and 11 post-conditioning. Rats were later re-exposed to predator odor-paired context alone and tested for CPA and alcohol drinking. Subsequently, rats were tested for compulsivity of alcohol drinking (willingness to consume quinine-flavored alcohol), and also mechanosensitivity (Von Frey test) in the absence and presence of predator odor. At the end of the experiment, all rats were re-exposed to the predator odor-paired context and sacrificed immediately, and brains were removed for Western blot analysis of extracellular signal-related kinase (ERK) and phosphorylated ERK (pERK) expression.
Results: Relative to non-avoiders and controls, avoiders exhibited consistently higher avoidance of the predator-paired chamber, higher operant alcohol responding, and more compulsive-like alcohol drinking. In the presence of predator odor, all rats exhibited allodynia, but this effect was greater in avoiders. Following

re-exposure to predator-paired context, avoider and non-avoider rats exhibited bi-directional changes in pERK expression relative to controls. Avoiders had increased, and non-avoiders decreased, ERK phosphorylation in infralimbic cortex relative to controls. Considering the role that infralimbic cortex has been attributed in fear conditioning processes, these changes in ERK phosphorylation may reflect either short- or long-term differences in the efficacy of fear extinction in these two groups of rats.
Discussion: This study presents a novel animal model for assessing the effect of a traumatic stressor and the maladaptive response to that stressor on subsequent alcohol drinking and related behaviors. This model may be useful for examining the underlying neural mechanisms of excessive alcohol consumption in humans with PTSD.
Disclosure: N. Gilpin: None. **S. Edwards:** None. **G. Koob:** Part 1: Addex Pharmaceuticals Alkermes Arkeo Pharmaceuticals Casa Palmera Embera NeuroTherapeutics GlaxoSmithKline Lilly Psychogenics.

11. Norepinephrine Transporter A457P Knockin Mice display Anxiety-Like Behavior and Tachycardia

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Background: Norepinephrine (NE) serves as a neurotransmitter in both the brain, where it mediates processes underlying arousal, attention, memory, emotion, and the response to stress, and in the sympathetic nervous system. NE neurotransmission is involved in the pathogenesis of cognitive and mood disorders including attention deficit hyperactivity disorder (ADHD), major depression and anxiety as well as cardiovascular diseases. NE released at synapses in the brain and sympathetic nervous system is actively transported into terminals by the presynaptically-localized norepinephrine transporter (NET), making NET a critical mediator of NE inactivation and presynaptic catecholamine homeostasis. NET is a target for tricyclic antidepressants, NET-selective reuptake inhibitors, and psychostimulants. We previously identified a single nucleotide polymorphism in the human NET gene, A457P, in a family with incidence of the cardiovascular disorder, postural orthostatic tachycardia syndrome, demonstrating highly elevated heart rate and plasma NE upon standing as well as psychiatric symptomatology. *In vitro* expression studies demonstrate that A457P is a loss-of-function transporter with a dominant-negative influence on wild-type (WT) NET. We generated "knockin" mice expressing NET A457P to determine the contribution of this mutation to both psychiatric and cardiovascular phenotypes.
Methods: For NE transport assays, synaptosomes and peripheral tissues were incubated for 10-30 min with 10 nM-1 microM [^{3}H]NE and specific activity was defined using 10 microM desipramine. We performed cell-surface biotinylation and immunoisolation from synaptosomes followed by SDS-PAGE and Western blotting with an antibody to NET (Mab Technologies). Catecholamines and metabolites were measured by high performance liquid chromatography from plasma and urine samples. Blood pressure radiotelemetry was used to determine blood pressure and heart rate in conscious mice. Mice were also exposed to the elevated plus maze for 5 minutes, and time spent/number of entries into the open versus closed arms and freezing behavior were measured using video analysis (Anymaze). Open field exploration was performed for 1 h during which detection of infrared beam breaks recorded movement. Total distance traveled and time spent in the center versus periphery of the field were calculated.
Results: In cortex and hippocampus of A457P heterozygous (HET) knock-in mice, total levels of NET were reduced to 69 and 73%, of WT, and surface levels of NET were reduced to 71 and 80% of WT, respectively. Transport levels in A457P HET mice were 61 and 63% of WT in cortex and hippocampus, respectively. These are greater transport deficits than that observed in NET heterozygous knockout mice, suggesting an *in vivo* dominant negative effect of A457P. In the heart and other peripheral, sympathetically-innervated tissues, NE transport was also reduced in A457P HET mice. Basal urine DHPG levels were decreased, NE was elevated, and the DHPG to NE ratio, an index of efficiency of metabolism to DHPG, was decreased in A457P mice, and similar results were observed in plasma. Radiotelemetry recordings demonstrated higher heart rates in A457P mice compared to WT mice, with the greatest effect occurring during their active period (648 ± 8 versus 589 ± 4 bpm). Mean blood pressure was not different, reminiscent of the predominant effect of A457P on heart rate in human carriers. A457P mice spent decreased time in the open arms and increased time in the closed arms of the elevated plus maze (EPM). A457P mice froze more often and for longer durations than WT mice and demonstrated decreased head dips in the EPM, all evidence of anxiety. We also detected anxiety in the open field, where A457P mice spent significantly less time in the center of the field and increased time along the periphery (thigmotaxis).
Discussion: These data support that A457P is trafficking- and activity-deficient in neurons *in vivo* and exerts a dominant negative effect. These data also demonstrate that A457P is sufficient to recapitulate the neurochemical and cardiovascular phenotypes observed in human carriers. Additionally, A457P mice demonstrate anxiety phenotypes. Indeed, patients with tachycardia disorders have a higher incidence of anxiety. Thus, genetic disruption in genes, such as NET, with shared expression in the brain and autonomic nervous system, may explain comorbidity of psychiatric and cardiovascular disease.
Disclosure: J. Shirey-Rice: None. **R. Klar:** None. **S. Redmon:** None. **J. Krueger:** None. **N. Wallace:** None. **M. Appasamy:** None. **C. Finney:** None. **S. Lonce:** None. **A. Diedrich:** None. **D. Robertson:** None. **M. Hahn:** None.

12. Altered Noradrenergic Activity In An Animal Model Of Post-Traumatic Stress Disorder

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Background: Locus coeruleus (LC) norepinephrine (NE) neurons are critical for coordination of the stress response and altered noradrenergic activity has been implicated in psychiatric disorders, including post traumatic stress disorder (PTSD). Norepinephrine is important for mediating arousal, attention, and memory processes and therefore altered NE function has been suggested as a possible contributor to both hyperarousal and re-experiencing symptom clusters of the disorder. In order to directly assess LC-NE system function following stress/trauma, we utilized a validated rodent model of PTSD - Single Prolonged Stress (SPS) – measured single unit activity of LC neurons and quantified TH mRNA expression in the LC.
Methods: 49 male Sprague Dawley rats were subjected to either SPS or a control procedure. SPS rats received two hours of restraint, followed by a twenty minute forced swim. After fifteen minutes recuperation, they were exposed to ether until anaesthetized, followed by a seven day quiescent period. Control rats remained in their home cages for the duration of this procedure. For electrophysiological recording ($n = 19$), rats were anaesthetized, LC neurons of stable amplitude were identified and spontaneous and sensory evoked discharge of LC neurons was

characterized. In a separate experiment (n = 32), baseline and restraint stress evoked TH mRNA expression were measured in the LC.
Results: SPS significantly reduced spontaneous discharge of LC neurons [$t(94) = 2.34$, $p = 0.022$] while significantly increasing the magnitude of the sensory evoked response [$t(94) = 2.79$, $p = 0.006$] relative to the control procedure, resulting in a significantly higher signal-to-noise ratio in SPS rats [$t(94) = 5.62$, $p < 0.0001$]. Similarly, TH mRNA expression in the LC was exaggerated in SPS rats following restraint stress compared to control rats [$F(1,27) = 4.63$, $p = .040$]. Percent increase (from mean baseline scores) in TH mRNA expression following restraint was significantly greater in SPS than in control rats [$t(14) = 3.21$, $p = .006$].
Discussion: These data demonstrate persistent changes in LC-NE system function stress/trauma and provide a framework for investigating noradrenergic changes in an animal model of PTSD. We report that both tonic and phasic LC activity are altered by SPS. Recent models of LC activity propose that different modes of LC discharge facilitate distinct processes (Aston-Jones and Cohen, 2005) with phasic activity mediating focused attention and tonic activity mediating attention to irrelevant stimuli, thereby facilitating task disengagement and environmental scanning. These data suggest that SPS animals may have altered LC activity that promotes increased reactivity to specific environmental events, and reduced behavioral flexibility.
Disclosure: S. George: None. **D. Knox:** None. **A. Curtis:** None. **R. Valentino:** None. **I. Liberzon:** None.

13. Analysis of Norepinephrine Dynamics within the Bed Nucleus of the Stria Terminalis in Rat Models of Substance Abuse and Post-Traumatic Stress Disorder

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Background: Stressful life experience often contributes to the pathology of several mental disease states including depression, anxiety disorders and addiction. Recent studies have highlighted the extreme co-morbidity of Post-Traumatic Stress Disorder (PTSD) and substance abuse. This has led researchers to focus on common neural substrates that are involved in mediating behaviors related to both stress and addiction. One such region is the Bed Nucleus of the Stria Terminalis (BNST), a component of the extended amygdala that relays between cognitive and emotion generating centers, and classical stress and reward nuclei. Furthermore this region receives one of the densest projections of norepinephrine (NE) within the central nervous system. Noradrenergic modulation within the BNST: induces synaptic plasticity, releases extra-hypothalamic corticotropin releasing factor (CRF), regulates stress-induced reinstatement to drug seeking, anxiety-like behavior and hypothalamic-pituitary-adrenal axis activation. Additionally, noradrenergic projections stemming from the nucleus of the tractus solitarius (NTS) are necessary for mediating conditioned place preference to morphine. These studies lead to the hypothesis that dysregulation of the noradrenergic system and its signaling within the BNST may be a component of the psychopathology of both PTSD and drug addiction, and may contribute to the co-morbidity observed within the human population. To investigate this hypothesis we examined the noradrenergic system in a rat model of PTSD and substance abuse, the Lewis (LEW) inbred rat strain as compared to the outbred Sprague-Dawley strain (SP-D). Aspects of dopaminergic neurons in naïve LEW rats are comparable to opiate dependent SP-D rats. Recent studies have also demonstrated that the BNST mediates pathological anxiety and fear responses in the LEW rats. Additionally, microdialysis experiments have demonstrated that LEW rats display a more robust NE response in the BNST to an acute stressor as compared to SP-D rats.
Methods: Here we utilize High Performance Liquid Chromatography with electrochemical detection (HPLC-ECD) and Fast-Scan Cyclic Voltammetry (FSCV) to measure NE dynamics within the ventral BNST (vBNST) of naïve LEW and SP-D rats.
Results: As compared to SP-D rats, we find that LEW rats have double the tissue content of NE in the vBNST as measured by HPLC (LEW rats: 10.86 ± 1.52 μg/g, SP-D: 4.55 ± 0.84 μg/g $p < 0.01$). Furthermore, we used Michaelis-Menten kinetic modeling of FSCV to examine sub-second stimulated release and uptake dynamics in the vBNST of anesthetized rats. LEW rats had a comparable [NE] per stimulation pulse to SP-D rats, but they demonstrated a slower rate of transport (0.189 ± 0.012 μM/s vs. 0.354 ± 0.042 μM/s respectively, $p < 0.001$). Additionally, when challenged with 5 mg/kg idazoxan (and α_2-adrenergic receptor antagonist) LEW rats had an attenuated increase in their stimulated NE release (LEW: 157.8 + 19.3% of baseline; SP-D: 215.9 + 20.7%; $p < 0.05$).
Discussion: The data presented here suggest that naïve LEW rats exhibit a hyper-adrenergic phenotype as compared to SP-D rats. This phenotype may contribute to both the increased self-administration of substances of abuse and the PTSD phenotypes observed in these animals. Interestingly, the Morilak group did not observe difference in basal extracellular NE in the BNST in LEW and SP-D rats, but observed enhanced NE following restraint stress in the LEW rats. An increase in NE tissue content with diminished uptake and auto-receptor function suggests that under conditions of prolonged firing during continual stressors LEW rats may release greater amounts of NE that may exert its effects for a longer period of time. This is particularly interesting in the BNST where the induction of noradrenergic plasticity is dependent on the length of exposure to NE. The changes observed here, however, may be but one mechanism accounting for increased adrenergic tone in these animals. Future studies will examine *in vivo* NE in animals performing in behaviorally relevant conditions to assess contributions of behaviors on NE release in the BNST.
Disclosure: Z. McElligott: None. **P. Walsh:** None. **R. Wightman:** None.

14. Reelin mediates the Neurosteroid-Induced Long-Lasting Improvement of Aggression and Anxiety-Like Behavior in a Mouse Model of PTSD

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Background: Reelin is a large glycoprotein widely expressed in GABAergic neurons and secreted into the extracellular matrix where it exerts important trophic functions at postsynaptic dendritic spines. Recently, an impairment of cognitive function in psychiatric disorders has been related to reelin deficits.
In a putative mouse model of PTSD, behavioral dysfunction, including heightened anxiety-like behavior and aggression, are induced by a protracted period of social isolation (< 4 weeks). Social isolation induces several neurochemical alterations in mice, including a downregulation of reelin expression and of the neurosteroid allopregnanolone (Allo), a progesterone metabolite, which is a potent positive allosteric modulator of GABA action at $GABA_A$ receptors. It has also been implicated in regulating neurotrophic actions. Hence, we hypothesized a link in the deficit of Allo and reelin biosynthesis that is associated with aggression and anxiety-like behavior in socially isolated (SI) mice. Both Allo

levels and reelin mRNA expression are downregulated in the medial frontal cortex (-35%), hippocampus (-55%), and amygdala (-65) but not in the striatum and cerebellum of SI mice.
Methods: To test whether a corticolimbic Allo deficit was responsible for the downregulation of reelin expression in SI mice, we administered Allo (8 μmol/kg, s.c., twice daily, for 7 days) or the selective brain steroidogenic stimulant (SBSS), S-norfluoxetine (1.8 μmol/kg, s.c., twice daily, for 7 days). This S-norfluoxetine dose is too low to inhibit serotonin reuptake (*Curr Opin Pharmacol* 9, 24-30, 2009) but is sufficient to increase the levels of Allo in corticolimbic areas to the levels of group-housed mice.
Results: This treatment successfully induced a long-term normalization of behavior and reelin mRNA expression in corticolimbic areas, including the frontal cortex, hippocampus, and amygdala of SI mice. Importantly, a single (25-1 ng/ml) bilateral microinfusion of reelin into the BLA permanently (up to 1 month) abolished anxiety-like behavior and aggression in SI mice in the absence of locomotion impairment.
Discussion: These observations suggest that by increasing neurosteroids, SBSSs such as S-norfluoxetine regulate reelin expression in corticolimbic areas and induce permanent improvement of some behavioral dysfunctions that relate to PTSD.
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Disclosure: M. Nin: None. **L. Martinez:** None. **M. Nelson:** None. **G. Pinna:** None.

15. Differential Role of ΔFosB in the Prefrontal Cortex in CCK Sensitivity and Vulnerability to Stress

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Background: Chronic social defeat stress in mice induces long-lasting abnormalities, including social avoidance, which can be normalized by chronic, not acute, antidepressant treatment (Berton *et al.*, 2006). However, a significant proportion of defeated mice avoid these abnormalities (Krishnan *et al.*, 2007), allowing a look at mechanisms of resilience in addition to vulnerability. Recently, we observed decreased expression of markers of neuronal activity in the medial prefrontal cortex (mPFC) of susceptible mice (Covington *et al.*, 2010), and found that optogenetic stimulation of the mPFC of susceptible mice reversed their depression-like symptoms (Covington *et al.*, 2010). Moreover, cholecystokinin (CCK) release in the mPFC during social stress mediates anxiogenic response, suggesting that it might contribute to the susceptible phenotype.
Methods: We quantified ΔFosB levels by immunohistochemistry, a marker implicated in the persistent neuronal alterations induced by chronic exposure to stress. In order to evaluate its role in sensitivity to stress, we used viral-mediated gene transfer into different regions of the PFC. We used quantitative PCR to evaluate CCK-B receptor, a known target gene of ΔFosB, after chronic social defeat and after ΔFosB manipulation. Finally, we infused CI-988 (1 ng), a CCK-B antagonist, into the mPFC of susceptible mice and test them for their social interaction.
Results: We show here that social defeat induces long-term molecular adaptations differentially in susceptible vs. resilient mice in the mPFC as well as in the orbitofrontal cortex (OFC), another prefrontal region important in mood regulation. Resilient mice show a greater induction of ΔFosB in the OFC, while susceptible mice show greater induction in the mPFC. These results suggest that ΔFosB induction after chronic stress in these two brain regions regulates specific neural pathways that contribute to the susceptible and resilient phenotype, respectively. Preliminary evidence indicates that ΔFosB overexpression in the OFC reduces immobility in the forced swim test, an antidepressant-like effect. We are currently testing whether ΔFosB overexpression in the OFC of susceptible mice promotes resilience. Conversely, ΔFosB overexpression in the mPFC increased social avoidance induced by social stress, a pro-depressant effect. Microinfusion of CI-988 in the mPFC reversed the social avoidance induced by social defeat. We are currently testing the binding of ΔFosB at the CCK-B promoter gene after social defeat.
Discussion: Long-term alterations in the mPFC mediate the behavioral abnormalities observed after social defeat. We identified ΔFosB and its target gene, CCK-B, as mediator of the depressive-like phenotype induced by chronic stress. Blockade of CCK actions in the mPFC promotes social interaction. Conversely, increased ΔFosB levels in the OFC after social defeat promotes resilience, suggesting a differential role for ΔFosB in OFC and mPFC. Together, these experiments complement our earlier demonstration that ΔFosB acting in the nucleus accumbens reward circuitry mediates resilience and antidepressant responses (Vialou *et al.*, 2010), and will provide a better understanding of the role of ΔFosB in the pathophysiology of depression and its treatment.
Disclosure: V. Vialou: None. **D. Ferguson:** None. **E. Nestler:** None.

16. Manipulating Brain pH Alters Fear Memory Consolidation Via Acid-Sensing Ion Channel-1a

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Background: Novel therapies are needed for post-traumatic stress disorder (PTSD), which poses a significant threat to the health and well being of those afflicted. Opportunities for improving therapy might be discovered by identifying new ways to manipulate fear conditioning in rodents, which models the intrusive memories in PTSD. Recently we found that acid sensing ion channels (ASICs) and brain pH play a critical role in fear memory acquisition in mice. Here we tested the hypothesis that manipulating brain pH may also alter fear memory consolidation, the conversion of short-term fear memories to long term.
Methods: Wild-type mice and ASIC1a-null mice in a congenic C57Bl6/J background were fear conditioned to auditory cue or context. During the time window of fear memory consolidation, 1-4 hrs post training, brain pH was lowered by 10% CO_2 inhalation for 30 minutes or raised by HCO_3^- injection (2 mmol/kg, i.p.). Fear memory was tested 24 hrs later by re-exposing the mice to the conditioned stimuli.
Results: We found that lowering brain pH by CO_2 inhalation potentiated fear memory consolidation in wild-type mice, but not in ASIC1a-null mice. This effect was observed with both context and auditory cue fear conditioning, but did not occur in a non-fear-related spatial memory task. CO_2 inhalation outside of the 1-4 hr time window failed to produce the effect. Other stressors such as restraint stress also failed to produce a CO_2-like effect. Importantly, raising brain pH with HCO_3^- during the consolidation window produced the opposite effect, inhibiting fear memory in an ASIC1a-dependent manner.
Discussion: These observations suggest that brain pH through its effects on ASIC1a plays a critical role in fear memory consolidation in mice. Because of the near-identical conservation of ASICs in humans, these results further suggest that brain pH may be a critical factor in human fear memory consolidation and the intrusive fear memories in PTSD. Moreover, the inhibitory effect of raising brain pH suggests a novel therapeutic approach that if applied shortly after an emotional trauma may help prevent PTSD.
Disclosure: J. Wemmie: None.

17. Role of Serotonin 2A Receptor Signaling During Development in Modulating Adult Affective Behavior

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Background: Serotonin 2A receptor (htr2a) function has been linked to several neuropsychiatric disorders. Elevated htr2a signaling correlates with vulnerability to stress, and htr2a levels are increased in postmortem brains of depressed patients. In contrast, htr2a antagonists exhibit both antidepressant and antipsychotic properties, and low htr2a expression has been associated with antidepressant efficacy in patients, and reduced anxiety in animal models. Because depression and anxiety disorders can have their origin early in life, we hypothesized that abnormal activation of htr2a during development may be detrimental to affective behaviors. We investigated this hypothesis in a mouse model in which transient blockade of the serotonin transporter (SERT) during the early postnatal period (p2-p11), induces "depression-like" affective behaviors in adulthood. The exact mechanism underlying how this pharmacological intervention induces depression-like states is unclear. We observed that htr2a mRNA is highly expressed in the cortex and hippocampus during postnatal stages, as compared to adult levels. This prompted us to ask whether enhanced postnatal activation of htr2a contributes to the adult behavioral phenotypes induced by early life SERT blockade.
Methods: To answer this question we studied affective behaviors in mice constitutively lacking htr2a and wild-type littermates, treated with postnatal fluoxetine (PNFlx) or vehicle (PNVeh). We tested subjects in several tests: (1) open field activity, (2) novelty suppressed feeding and (3) shock-avoidance test. These are all paradigms in which wild type PNFlx mice display anxiety and depression-related phenotypes.
Results: We found that the absence of htr2a improved performance of PNFlx treated mice in the novelty suppressed feeding task, by decreasing the latency to feed, to control levels. Absence of htr2a, however, did not have ameliorative effects on PNFlx phenotypes in the open field and shock avoidance tests. In an independent cohort, we tested htr2a knockout mice in a learned helplessness paradigm, a test in which PNFlx wild-type mice show increased "helplessness." We found that htr2a deficient mice were less helpless, with reduced latencies to escape compared to wild-type mice.
Discussion: These results indicate that htr2a is responsible for the conflict-anxiety phenotypes observed in PNFlx mice but does not contribute to the changes in exploratory behavior and shock avoidance. Our findings also raise the possibility that the increased learned helplessness in PNFlx mice could be another aspect that is dependent on htr2a function.
Disclosure: E. Demireva: None. **Q. Yu:** None. **J. Muller:** None. **M. Ansorge:** None. **J. Gingrich:** None.

18. The Role of Cortical Norepinephrine in the Development of Executive Function

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Background: Adolescent rats are less able than adult rats to learn reinforcement reversals and shift attentional set. These two forms of executive function rely on the functional integrity of the orbitofrontal and prelimbic cortices, respectively. Drugs used to treat attention deficit disorder that increase prefrontal catecholamine levels such as atomoxetine (Strattera) improve executive functions in humans, non-human primates and adult rats with noradrenergic lesions of the prefrontal cortex. Cortical noradrenergic systems are some of the last to mature in primates and rats. This maturational lag is hypothesized to underlie the immaturities in executive function found in adolescents.
Methods: We assessed executive function in male, Long-Evans rats using an intradimensional/extra-dimensional set shifting task. We administered the norepinephrine transporter (NET) blocker, atomoxetine (0.0, 0.1, 0.9 mg/kg/ml; i.p.), prior to the test of attentional set shift and a reinforcement reversal. We also assessed in the attentional set shifting task the efficacy of drugs that either selectively block dopamine transporters (DAT; GBR 12909 0.0, 0.3, 3.0 mg/kg/ml; i.p.) or block both NET and DAT (methylphenidate; 0.0, 0.5, 2 mg/kg/ml) to determine if increasing synaptic dopamine would improve cognitive performance. In addition, we completed stereological analyses to determine how the density of dopamine beta hydroxylase and NET changed over the course of adolescence i.e. between post-natal day (PND) 40 and PND 50.
Results: The ability to shift attentional set improves from early to late adolescence. The lowest dose of atomoxeting facilitated attentional set shifting during early adolescence but had no effect on reversal learning. These data demonstrate that NET blockade allows adolescent rats to more easily perform attentional set shifting. Stereological analyses showed that NET density is higher at PND 40 than PND 50 in all prefrontal subregions sampled including prelimbic, infralimbic, anterior cingulate and orbitofrontal cortices. Dopamine beta hydroxylase density is lower in these same regions at PND 40 when compared to PND 50.
Discussion: We hypothesize that lower levels of synthetic enzyme in combination with higher density of presynaptic transporters leads to a functional deficiency in prefrontal noradrenergic systems and underlies the immaturities in executive function. Young adolescent rats, e.g. post-natal day 43 (PND 43), require more trials to shift attentional set than adults.This difference between adolescent and adult executive function is not present in late adolescence (PND 50). The administration of a NET blocker which prevents reuptake of norepinephrine is sufficient to improve attentional set shifting in young adolescents. As NET blockade improves cognitive performance, it supports the hypothesis that post-synaptic function is similar in adults and adolescents but future studies will be aimed at addressing this question.
Disclosure: K. Agster: None. **A. Bates:** None. **R. Cain:** None. **L. Newman:** None. **B. Waterhouse:** None. **J. McGaughy:** None.

19. Poster Withdrawn

20. Elevated Glutamate Levels in the Striatum, Nucleus Accumbens Core and Prefrontal Cortex of the Spontaneously Hypertensive Rat Model of ADHD

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Background: Attention-deficit/hyperactivity disorder (ADHD) is theorized to be a disorder of catecholamine dysfunction; however,

recent evidence suggests that glutamate may play some role. Clinical studies of children and adults with ADHD show increased levels of glutamate/glutamine in the striatum and prefrontal cortex and studies of signaling interactions between the dopamine and glutamate systems demonstrate that dopamine D2 receptors are involved in downstream inhibition of the NMDA receptor. Our group has previously shown that D4DR knockout mice have lowered basal ganglia DA and elevated glutamate in the prefrontal cortex. We have recently shown that the spontaneously hypertensive rat model of ADHD, combined type (SHR/NCrl – from Charles River line) has decreased evoked dopamine release in the dorsal striatum versus a model of ADHD inattentive type (WKY/NCrl) and faster dopamine uptake in the striatum and nucleus accumbens core in the SHR/NCrl versus control (WKY/NHsd). For this study, we hypothesized that altered dopamine dynamics in the striatum may be leading to more active NMDA receptor signaling resulting in increased glutamate output in the striatum, nucleus accumbens, and prefrontal cortex in the SHR rats.

Methods: Tonic and KCl-evoked release of glutamate were investigated in the striatum, nucleus accumbens, and prefrontal cortex of 8 week old anesthetized WKY/NHsd, WKY/NCrl, and SHR/NCrl.We utilized an enzyme-based microelectrode array (MEA) that was selective for glutamate measures with fast temporal (2 Hz) and high spatial (15 x 333 μm) resolution. A two-way analysis of variance followed by a Bonferonni post-hoc test was used to analyze resting and KCl-evoked glutamate levels.

Results: SHR rats showed higher tonic and KCl-evoked release of glutamate in all sub-regions of the striatum (vs. control/ WKY/NCrl: tonic – dorsal 230%/305%, intermediate 330%/530%, ventral 330%/330%: evoked – dorsal 150%/130%, intermediate 265%/320%, ventral 240%/315%), the core of the nucleus accumbens (vs. control/ WKY/NCrl: tonic – 295%/375%: evoked – 270%/210%) as well as the prefrontal cortex (vs. control/ WKY/NCrl: tonic – prelimbic 230%/150%, infralimbic 225%: evoked – prelimbic 230%/530%, infralimbic 315%) – all areas highly implicated in ADHD ($p<0.05$). Therefore SHR rats displayed increased levels of glutamate in all regions compared to the WKY/NHsd control. SHR rats showed significant increase in glutamate levels in the most areas when compared to the WKY/NCrl model of inattentive type ADHD, except for the infralimbic prefrontal cortex, which was significantly elevated in both models when compared to control (WKY/NHsd).

Discussion: We have demonstrated elevated glutamate dynamics in the prefrontal regions of SHR rats which correlates with similar findings in D4DR knockout mice. These findings suggest that glutamate regulation may also be a potential target for pharmacotherapy in ADHD.

Disclosure: P. Glaser: None. **E. Miller:** None. **G. Gerhardt:** Part 1: Quanteon Inc., Part 4: Eli Lilly.

21. Haploinsufficiency of the Intellectual Disability and Autism susceptibility gene, *SynGAP1,* causes Premature Excitatory Synapse Development and Neonatal Hippocampal Dysfunction

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Background: Disruptions to the molecular mechanisms controlling glutamatergic synapse structure and function are believed to underlie certain neurodevelopmental disorders, such as Intellectual Disability (ID), Autism Spectrum Disorder (ASD) and schizophrenia. Deleterious mutations in several synaptic proteins have been liked to these disorders, though the impact of such mutations on neural development and cognitive maturation is unclear. Recently, haploinsufficiency of the gene that encodes the essential synaptic RasGAP, SynGAP, was shown to cause ID in 3-4% of sampled patients. CNVs of this gene are linked to ASDs and SynGAP expression levels are altered in elderly patients with schizophrenia. Because SynGAP is exclusively localized to forebrain dendritic spines, where it directly regulates G-protein signaling controlling glutamatergic synapse structure and function, the neurodevelopmental outcome of *SynGAP1* mutations may provide valuable insight into the patho-neurobiology underlying these disorders.

Methods: The mice used for in this study were a mixed genetic background of 129sv/ev (Taconic) and c57/B6J (Jackson Labs). Electrophysiological studies were carried out in mice at three different age groups: PND8-9, PND14-16, PND > 60. Two-photon imaging of dendritic spines in acute slices was performed by exciting eGFP in SynGAP Het mice crossed to THY1-GFPm mice. Laser photo-stimulation was performed by uncaging glutamate in the dentate gyrus and then measuring spread of activity using a voltage sensitive dye. Dentate gyrus function was assessed by mouse performance in a memory test designed to to test pattern separation.

Results: We show that excitatory synapses in the hippocampus of *SynGAP1* haploinsufficient mice develop at an accelerated rate starting in the second postnatal week. The changes in synaptic function were specific to glutamatergic synapses and were caused by elevated AMPAR function. There were no observed alterations to intrinsic neuronal properties or inhibitory currents onto glutamatergic neurons. At this same point in development, SynGAP mutants exhibited enhanced excitability of the hippocampal tri-synaptic circuit. Laser photo-stimulation of the dentate gyrus resulted in a more than 8-fold increase in signals reaching CA1 from Hets compared to WT mice. In addition, SynGAP P16 SynGAP Hets also exhibited an enhanced seizure threshold and audiogenic seizures, while also exhibiting behavioral abnormalities related to hippocampal dysfunction. These developmental disruptions had persistent consequences because adult mutants performed poorly on a test for pattern separation, a memory test designed to assess hippocampal function during memory encoding and retrieval.

Discussion: We conclude that SynGAP expression restricts the functional maturation of glutamatergic synapses by tempering AMPAR accumulation at postsynaptic sites. This negative tuning of glutamatergic synapses by SynGAP appears essential for maintaining the balance of neural excitability in developing hippocampal networks, which has implications for the organization of connected brain regions and the emergence of cognitive ability. SynGAP tempers synaptic function by down-regulating signals in dendritic spines that promote plasticity. Thus, these studies provide a neurobiological link connecting a disease-causing genetic mutation to the abnormal development of neural circuits that underlie human intellectual ability.

Disclosure: G. Rumbaugh: None. **J. Chelliah:** None. **M. Aceti:** None.

22. Neurogenesis is not Required for Lithium's Effect in the Forced Swim Test suggesting that Lithium's Antidepressant-Like Effect is not Mediated via GSK-3 Inhibition

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Background: Despite six decades of use in the clinics and intensive research the molecular mechanism underlying lithium's mood stabilizing effect has not been unraveled. Among suggested hypotheses two central ones are the inositol depletion *via* inhibition of inositol monophosphatase and neuroprotection, mostly *via* inhibition of GSK-3. Chronic lithium treatment has

been demonstrated to increase dentate gyrus neurogenesis in adult rodents (Chen *et al*., *J Neurochem* 2000) to reduce mice immobility in the Porsolt forced-swim test (FST) model of depression and to attenuate amphetamine-induced hyperlocomotion model of mania. Bessa *et al*. (*Mol Psychiatry* 2009) showed that antidepressants retain their antidepressant-like effect in the FST even when neurogenesis is blocked by the cytostatic agent methylazoxymethanol (MAM). The DNA-alkylating agent temozolomide (TMZ) which penetrates efficiently the blood brain barrier and exhibits an advantageous side effect profile has also been shown to suppress neurogenesis in adult mice. We hypothesized that similarly to Bessa *et al*.'s finding blockade of neurogenesis will not affect the behavioral impact of lithium. Specifically, we studied whether lithium-induced decreased immobility in the FST and attenuated amphetamine-induced hyperactivity remain under neurogenesis-arrest conditions.
Methods: Four groups of mice (control, Li, MAM or TMZ and Li + MAM or Li + TMZ) were administered bromodeoxyuridine (BrdU) and studied behaviorally.
Results: Daily administration of 1.5 mg/kg MAM or 25 mg/kg TMZ for 14 days had no effects on general behavioral parameters. As expected, the regime of MAM or TMZ treatment reduced neurogenesis efficiently and lithium treatment reduced immobility in the FST but MAM or TMZ pretreatment did not block the latter effect.
Discussion: The results suggest that lithium's effect on neurogenesis is not involved in its antidepressant-like mechanism in the FST. Given ample evidence suggesting that lithium promotes neurogenesis *via* GSK-3β inhibition (Wexler *et al*., *Mol Psychiatry* 2008) it is plausible that lithium's antidepressant-like effect is not mediated *via* GSK-3β inhibition. Preliminary results in our lab show increased neurogenesis in homozygote knockout mice of the inositol transporter. Since these mice exhibit lithium-like reduced brain inositol levels and behavioral phenotype in the FST and the amphetamine-induced hyperactivity paradigm (Agam *et al*., *Biochem Soc Trans* 2009 and unpublished data)we suggest that inositol depletion rather than GSK-3 inhibition mediate lithium's mood stabilizing effects.
Disclosure: G. Agam: None. **N. Kara:** None. **S. Narayan:** None. **H. Einat:** None. **V. Vaidya:** None. **R. Belmaker:** None.

23. HDAC6 Regulates GR Signaling in Serotonin Pathways with Critical Impact on Stress Resilience

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Background: Genetic variations in certain components of the glucocorticoid receptor (GR) chaperone complex have been associated with the development of stress-related affective disorders and with individual variability in therapeutic responses to antidepressants (ATD). Mechanisms that link GR chaperoning to stress-related disorders are not well understood. Here, we demonstrate that the effects of glucocorticoid hormones on affective behaviors are critically regulated via reversible acetylation of Hsp90, a key component of the GR chaperone complex. We provide pharmacological and genetic evidence indicating that the cytoplasmic lysine deacetylase HDAC6 controls Hsp90 acetylation in the brain and thereby modulates Hsp90-GR protein-protein interactions and GR downstream signaling, with a critical impact on stress resilience.
Methods: 1) We conducted an analysis of gene expression profiles associated with resilience and ATD responses in raphe nuclei. After 10 days of social defeat (SD), experimental mice were stratified into stress resilient or stress vulnerable subpopulations based on a measure of social avoidance. Cohorts were subsequently treated with imipramine or vehicle for 28 days. Microarrays and qPCR were used to quantify gene expression changes. Fluorescently tagged serotonin (5-HT) neurons were enriched by LCM from Epet-YFP midbrain tissues. This transcriptome analysis identified HDAC6 as one of the most robustly regulated gene. The ability of a wider range of ATD from various chemical classes to regulate HDAC6 expression was then examined under cell culture conditions using the RN46 immortalized raphe neuronal cell line. Distribution of HDAC6 mRNA and protein was also mapped across the entire mouse brain using in ISH and IHC. 2) We used a HDAC6 loss of function strategy to examine the influence of this gene on stress vulnerability and on morphological and electrophysiological characteristics 5-HT neurons. Cre-lox mediated recombination was used to generate 2 lines of mice harboring a pan-neural or serotonin-specific conditional depletion of HDAC6. These mice were subjected to an extensive behavioral profiling comprising several measures of depression-related behaviors. Under baseline conditions and after 10 days of SD, whole cell recordings and morphological analyses in biocytin-filled 5-HT neurons were also conducted from midbrain slice in WT and HDAC6 deficient mice.
3) We examined the ability of HDAC6 to regulate downstream GR signaling and modulate behavioral and neurophysiological effects of glucocorticoid hormones. Hsp90 acetylation and Hsp90-GR protein-protein interactions were examined using Hsp90 IP followed by WB. GR nuclear translocation was examined in response to SD *in vivo* and following Dexamethasone treatment in cell culture. Expression of GR target genes was examined by qPCR.
Results: HDAC6 was highly enriched in serotonin neurons of the Raphe. A significant albeit lower density HDAC6 immunopositive cells was also observed in the hippocampus and lateral septum. HDAC6 in raphe cells was consistently downregulated after administration of ATDs from various chemical classes. Downregulation of HDAC6 also occurred spontaneously after SD in stress resilient but not stress vulnerable mice. Selective loss of HDAC6 in serotonin neurons was devoid of behavioral effects in naïve mice but promoted ATD–like responses in mice exposed to inescapable stress, reducing social avoidance in the SD paradigm and decreasing immobility in the FST and TST. Serotonergic depletion of HDAC6 also prevented SD-induced Hypoexcitability and SD-induced hypertrophy of 5-HT neurons, 2 changes occurring robustly in stress-vulnerable WT mice. Conditional deletion of HDAC6 resulted in the hyperacetylation of α-tubulin and Hsp90, but had no effect on acetylation of Histone H3. Hsp90 hyperacetylation was associated with a decreased association between Hsp90 and GR. Both pharmacological inhibition and genetic depletion of HDAC6 antagonized hormone- and stress -induced nuclear translocation of GR in raphe neurons and prevented transcriptional, electrophysiological and pro-depressant effects of exogenously administered glucocorticoids.
Discussion: Our results identify HDAC6 as a novel target for pro-resilience and ATD interventions via focal prevention of GR signaling in serotonin pathways. Our data also uncover a fundamentally novel mechanism by which pan-HDAC inhibitors may regulate stress-related behaviors independently of their actions on histones.
Disclosure: J. Espallergues: None. **S. Teegarden:** None. **S. Beck:** None. **O. Berton:** None.

24. Linking Depression to Inflammation through Dysregulated Glycogen Synthase Kinase-3 (GSK3)

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Background: Accumulating evidence shows that inflammation strongly influences the development and treatment of depression,

a debilitating disease with a lifetime incidence of ~20%. Inflammatory molecules are elevated in many patients with depression, administration of inflammatory cytokines induces depression in susceptible people, and inflammation in rodents causes depressive-like behaviors and impairs antidepressant therapeutic effects. We identified glycogen synthase kinase-3 (GSK3) as an important regulator of the immune system that could contribute to its action in promoting susceptibility to mood disorders.Previous links of GSK3 to mood disorders include: (a) GSK3 is inhibited by mood stabilizers and antidepressants, (b) pharmacologicalor geneticreduction of GSK3 activity reduces depression-like behaviors in rodents, (c) GSK3 is activated when serotonergic signaling is deficient, (d) neurotrophins such as brain-derived neurotrophin factor that may be deficient in depression normally inhibit GSK3, and (e) studies of human serum, postmortem brain, and polymorphisms implicate GSK3 in mood disorders.

Methods: GSK3 activity was increased by using GSK3$\alpha/\beta^{21A/21A/9A/9A}$ knockin mice with serine-to-alanine mutations to block inhibitory serine-phosphorylation of GSK3 or was decreased by administration of GSK3 inhibitors (lithium, CHIR99021 and TDZD-8). Levels of inflammatory cytokines were measured in supernatants of cultures of primary astrocytes and microglia, in $CD4^+$ T cells, or in brains of mice subjected to immune challenges. Activation of the transcription factor signal transducer and activator of transcription-3 (STAT3) was assessed in astrocytes, $CD4^+$ T cells and brain. The learned helplessness paradigm of depression-like behavior was evaluated in adult male mice.

Results: Inhibition of GSK3 or knockdown of GSK3 inhibited inflammatory cytokine production by astrocytes and microglia and blocked the inflammatory activation of the transcription factor STAT3. Blocking cytokine production and downregulation of STAT3 by GSK3 inhibition reduced the differentiation of T cells towards pathogenic Th17 cells, whereas active GSK3 promoted depressive-like behavior in mice and increased Th17 cells in mouse brain.

Discussion: Altogether, these findings indicate that GSK3 promotes inflammatory immune system activation and depression-like behavior. Activation by dysregulated GSK3 of the immune system may contribute to susceptibility to mood disorders and be controlled by mood stabilizers.

Disclosure: E. Beurel: None. **R. Jope:** None.

25. Native Immune System Regulation of the Brain Serotonin Transporter

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Background: Both the homeostasis of central serotonin (5-HT) signaling and immune system activation have been linked to psychiatric conditions such as depression, obsesive-compulsive disorder, and autism spectrum disorder. Dissecting the interaction between the serotonergic signaling and immune system activation may shed insight on these disorders and provide new therapeutic opportunities. The 5-HT transporter (SERT), which supports 5-HT inactivation and recycling, is tightly regulated by multiple signaling pathways including those initiated by receptor activation by the proinflammatory cytokines IL-1b and TNF-a. We have shown that these cytokines stimulate SERT activity in a p38 MAPK-dependent in raphe-derived cells *in vitro* and in mouse synaptosomes *ex vivo* (Zhu *et al*., 2006). Recently we demonstrated that IL-1 receptor (IL-1R) KO mice fail to display brain SERT stimulation following peripheral immunological challenges (Zhu *et al*., *Neuropsychopharmacology* 2010).

Methods: We examine SERT/IL-1R receptor/p38 MAPK colocalization by immunofluorescence. We examine IL-1R/SERT interactions through co-immunoprecipitations of co-transfected cells. We examine SERT phosphorylation using SERT immunopreciptation following IL-1R activation with IL-1b in SERT/IL-1R cotransfected cells. We use homologous recombination to develop floxed IL-1R mice. We use floxed p38 MAPK mice crossed to ePET-1 Cre animals to achieve raphe-specific elimination of p38 MAPK. Synaptosomal transport assays are used to assess SERT activity under basal conditions and following *in vitro* IL-1b stimulation or i.p. LPS injections.

Results: We provide evidence that IL-1R co-localizes with SERT in the dorsal raphe. In Chinese Hamster Ovary (CHO) cells transiently transfected with IL-1R and SERT, we find that SERT physically associates with IL-1R, and that SERT phosphorylation is enhanced by the treatment with IL-1b (100 ng/mL). Generation of animals with conditional raphe IL-1R deletion is underway to explore whether IL-1Rs expressed in serotonergic neurons is sufficient to mediate systemic LPS effects. Systemic injection of LPS produces enhanced phosphorylated p38 MAPK in serotonergic raphe neurons. We find that raphe p38 MAPK (both total and phosporylated) immunoreactivity is lost in animals bearing a raphe-specific deletion of p38 MAPK, and these animals fail to report LPS-induced SERT stimulation.

Discussion: Our results indicate that IL-1R and midbrain p38 MAPK are critical in immune stress-stimulated central SERT activity and suggest that changes in SERT activity induced by these pathways can contribute to altered presynaptic 5-HT homeostasis. Our studies indicate that p38 MAPK expressed in the raphe is critical to systemic immune system activation of SERT. Our biochemical findings support the possibility that IL-1R mediated activation of p38 MAPK and SERT phosphorylation supports SERT activation in serotonergic terminals. Studies are underway to examine how these signaling pathways impact 5-HT linked behaviors.

Disclosure: C. Zhu: None. **K. Lindler:** None. **N. Baganz:** None. **W. Hewlett:** None. **R. Blakely:** None.

26. Fewer Mature Neurons in the Anterior Dentate Gyrus in Major Depression: Rescue by Antidepressants

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Background: We previously reported that subjects with major depression (MDD) treated with antidepressants have more neural progenitor cells (NPCs) in the anterior dentate gyrus (DG), compared with untreated MDD and controls, although this effect is more pronounced before 45 years of age. We found no evidence of less neurogenesis in untreated MDDs; specifically there were not fewer neural progenitor cells (NPCs) in untreated MDDs compared with non-psychiatric controls of the same age and sex. Nevertheless, the maturation of NPCs may be altered in MDD and, similarly, antidepressants might affect neuron differentiation and maturation. Lesion studies in rats and functional studies in primates have shown a functional dissociation of the anterior and posterior DG. Therefore, we have now estimated the number of mature granule cells at three anteroposterior levels of the DG: uncal or anterior (*pes*), mid-body and posterior body, in five matched triplets of non-psychiatric controls, untreated MDDs and MDDs treated with selective serotonin reuptake inhibitors (MDD*SSRIs) for at least three months before death, confirmed by post mortem brain toxicology.

Methods: Tissue was obtained from the Macedonian/New York State Psychiatric Institute Brain Collection. All research was conducted with IRB approval. At autopsy, 2 cm-thick coronal blocks of the right hemisphere were flash-frozen in 1,1,1,2-tetrafluoroethane (-26.3C) and stored at −80°C. All subjects underwent psychological autopsy, brain pH determination, neuropathology and toxicology screens. Over 30 drugs, including antidepressants and drugs of abuse, were screened for and quantified in brain, blood and urine. Frozen tissue blocks of the whole hippocampus from the three groups (n = 5 each) were fixed and sectioned at 50 μm. Subjects were matched for age (within 10 years, 17-62 years inclusive), sex (females = 6, males = 9) and postmortem interval (PMI, within 10 hours, 4-24 hrs inclusive). Mature neurons were labeled by immunocytochemistry (anti-NeuN mouse monoclonal antibody, 1:100,000; Chemicon, Temecula, CA). The number of granule cells in the entire DG and in the three anteroposterior levels, were estimated by stereology (StereoInvestigator, MBF Biosciences Inc., Williston, VT), sampling was performed every two mm.
Results: Granule cell number differs between controls (69,595 ± 34,677; mean ± SD), MDDs (21,052 ± 7,993) and MDD*SRRIs (47,794 ± 32,395) in the anterior DG ($F = 4.229$, $df = 2,14$, $p = .041$). Untreated MDDs have fewer granule cells in the *pes* compared with controls ($p = 035$). We did not find differences in the number of granule cells in posterior DG. The number of granule cells and NPCs correlate robustly in the *pes* ($r = .714$, $p = .014$), mid body ($r = .737$, $p = .010$) and entire DG ($r = .698$, $p = .006$). The granule cell number in the whole DG correlated with the volume of the granule cell layer ($r = .775$, $p = .001$) and the volume of the DG (including all its layers: granule cell layer, molecular layer and subgranular zone, $r = .788$, $p = .001$).
Discussion: We found fewer granule cells in the anterior DG of untreated MDDs compared with non-psychiatric controls. In contrast, the number of granule cells in MDD treated with SSRIs was comparable to that of controls. In our previous study, we could not detect an effect of MDD on numbers of NPC or proliferating cells. Therefore, fewer mature granule cells in MDD could be due to a defect of maturation of NPCs or survival of granule cells in the DG. Alternatively, a small decrease in NPC number at a specific point in time may be harder to detect than the cumulative effect over a lifetime, which is what we assess when we count total granule cells. The smaller DG volume reported by some *in vivo* imaging studies in MDD may be the result of fewer neurons, dendritic atrophy or fewer glial cells. Our findings suggest that it could be related, at least in part, to fewer granule cells in MDD. *In vivo* hippocampal volume has been reported to increase with antidepressant medication in post-traumatic stress disorder and MDD. Our findings are correlational but suggest that SSRIs may normalize deficient NPC maturation and differentiation or granule cell survival in MDD. The difference in granule cell number between MDDs and controls was detected selectively in the anterior DG. Since the anterior hippocampus is reported to be more involved in emotion, the smaller number of granule cells in the anterior DG in MDD, may affect mood regulation.
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27. The Autism-associated Integrin Beta 3 Gene (*ITGB3*) Modulates the Behavioral and Neurochemical Response to Chronic Stress in Mice

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Background: Positive responses to stress involve adaptive changes that certain individuals utilize to cope with stressful events, including modifications in the neuromodulatory homeostasis, alterations in synaptic structure and function. The mechanisms by which these changes occur are not well understood, although roles for glucocorticoids, brain-derived neurotrophic factor and serotonin have been described. The integrin beta3 gene (*ITGB3*) has been repeatedly associated with autism and elevated serotonin blood levels (hyperserotonemia), although the mechanism by which integrin beta3 modulates brain development and function remains unknown. In the brain, integrin beta3 associates with the alphav subunit forming the vitronectin receptor. Integrin alphavbeta3 modulate the assembly of synapses through the recruitment of scaffolding proteins that dictate receptor localization and synaptic vesicle function. Recent discoveries linking beta3-containing integrins subunit with serotonergic systems led us to consider the hypothesis that alphavbeta3 modulates the response to chronic stress. Here we demonstrate that integrin beta3 signaling pathways are involved in chronic stress response.
Methods: We utilized a combination of biochemical, neurochemical and behavioral approaches to correlate alterations in integrin beta3 signaling and chronic stress. We chose the unpredictable chronic stress model (UCS) to trigger the stress response in mice. Mice were divided into two groups to assess the effects of chronic stress on depressive and anxiety related behaviors. The non-stressed group was housed with littermates, while individuals assigned to the stressed group were housed individually to determine the effects of social isolation. Mice assigned to the stressed group were exposed to one mild stress stimuli in a randomized fashion for a duration of 7 weeks to allow ample time for the development of depressive and anxious behaviors. Behavioral testing was conducted during the last week of the 7 week procedure, and mice were not exposed to stressors before behavioral testing. After the behavioral testing, mice were and euthanized for biochemical and neurochemical measurements.
Results: Our first cohort consisted in C57BL/6J mice exposed to the unpredictable chronic stress (UCS) model ($N = 7$) and control animals ($N = 5$). Biochemical analyses reveal that both subunits of the alphavbeta3 receptor are downregulated during chronic stress. As a compensatory mechanism, the downstream adaptor proteins talin and FAK are upregulated. These modifications correlate with latency to immobility and immobility time in the forced swim test, respectively. We then examined the behavioral and neurochemical responses in mice with reduced integrin beta3 expression (*Itgb3*). Our second cohort consisted on *Itgb3* heterozygous mice ($Itgb3^{-/+}$, $N = 14$) and wild-type littermates ($N = 13$). Stress-induced reductions in weight gain and nestlet shredding were observed in wild-type mice and absent in $Itgb3^{-/+}$ mice. $Itgb3^{-/+}$ mice also displayed reduced response to stress in the elevated zero maze and forced swim test, indicating that alphavbeta3 is involved in the behavioral response to chronic unpredictable stress in mice. Neurochemical analysis of brain tissue samples was conducted to provide an indirect measure of monoamine neurotransmission. Our findings demonstrate altered basal monoamine neurotransmission in the midbrain and neocortex as well as differential responses to chronic stress in $Itgb3^{-/+}$ mice relative to wild type controls.
Discussion: Here we identify a novel signaling pathway involved in the modulation of emotional reactivity in mice. Our studies demonstrate that integrin alphavbeta3 represents a novel mechanism by which the response to chronic stress is modulated. Both alphav and talin expression levels are correlated with immobility in the forced swim test, suggesting that reduced alphavbeta3 levels may influence the depressive response to chronic stress. Indeed, $Itgb3^{-/+}$ mice are resilient to stress as measured in the forced swim test. $Itgb3^{+/-}$ mice also exhibit increased basal anxiety and diminished stress-induced alterations in these behaviors. Neurochemical results indicate that integrin alphavbeta3 influences serotonergic metabolism and neurotransmission, which may

underlie observed differences in the behavioral responses. Given the roles of integrin alphavbeta3 in the modulation of the serotonin and glutamate systems, our further investigation of this novel network promises insights into the pathophysiology of depression and post-traumatic stress disorder.
Disclosure: S. Varney: None. **C. Gaskill:** None. **T. Jessen:** None. **A. Carneiro:** None.

28. Stimulation of the Innate Immune System is Accompanied by Modulation of Protein Expression and Phosphorylation State of Synaptic Markers in the Hippocampus

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Background: Clinical evidence suggests that pro-inflammatory cytokines play an important role in the pathology of Major Depressive Disorder (MDD). Elevated circulating pro-inflammatory cytokines can be found in depressed patients. Stimulation of the primary host defense system and immunotherapy such as is used in the treatment of multiple sclerosis has been shown to precipitate depressive symptoms. In addition, acute treatment with pro-inflammatory cytokines has been shown to increase glutamate function in the CNS. Thus inflammation may lead to a state of CNS glutamatergic hyperfunction such as that thought to exist in MDD. The studies described herein begin to elucidate the neuroadaptive mechanisms in the hippocampus associated with elevated circulating pro-inflammatory cytokines, induction of indoleamine-2,3-dioxygenase (IDO) and associated behavioral changes. We have treated CD-1 mice with BacilleCalmetteGuerin (BCG) to stimulate the innate immune response and examined hippocampal tissue from these animals for changes in protein expression and phosphorylation state of markers for a glutamatergic synapse.
Methods: Male CD1 mice 8- to 10-week were housed individually under a normal 12:12 h light:darkcycle. Food and water were available ad libitum. Mice were handled daily for at least 1 week before the onset of the experiment to minimize stress reactions to manipulation. All animal care and use were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NRC) and approved by the Institutional Animal Care and Use Committee. Mice were injected with saline or BCG (10^7 CFU/mouse in 200 ul, i.p.) a live attenuated vaccine for tuberculosis. All mice were weighed for 5 days before treatment and for 7 consecutive days after. Plasma cytokines were measured using the Luminex XMap technology. Total RNA was isolated from lung tissue, DNase-treated and used to synthesize cDNA for real-time RT-PCR using the TaqMan technology to assess changes in cytokine and IDO mRNA. Hippocampal tissue was processed in homogenization buffer and changes in total protein expression and phosphorylation state for GLUR1, NR1, and CREB and total protein expression of synaptophysin and PSD95 were assessed using polyacrylamide gel electrophoresis, transfer of proteins to nitrocellulose, hybridization with primary antibodies and the use of LiCor Infrared imaging. Beta-actin total protein was used to normalize the quantitative measure of the changes in protein expression and phosphorylation state.
Results: Transient body weight loss shortly after BCG inoculation together with an enlargement of the spleen, a significant increase in lung cytokine and IDO mRNA expression and elevation in plasma cytokines served as positive controls for BCG treatment efficiency in stimulating the innate immune response. Data from these studies confirm the development of an acute sickness behavior and are in agreement with data published by Moreau and colleagues. Hippocampal tissue from the above mentioned experiments was examined for changes in expression and phosphorylation state of synaptic markers as a measure of neuroadaptive changes at 9days post BCG treatment, a time point where a depressive phenotype has been reported previously. Our preliminary data show an increased expression of the AMPA receptor subunit GLUR1 ($207 \pm 34\%$ saline control, $p = 0.0086$), the post synaptic density protein PSD95 ($206 \pm 42\%$ saline control, $p = 0.03$), synaptophysin ($156 \pm 6.6\%$ saline control, $p = 0.0055$) and an increase in phosphorylation of GLUR1 Ser845 ($145 \pm 17\%$ saline control, $p = 0.0278$) at 9 days post BCG treatment. CREB total protein showed a trend toward an increase ($155 \pm 16\%$ saline control, $p = 0.08$) at the same time point but no significant change in CREB total protein or phosphorylation was detected. Neither total protein expression for the NMDA receptor subunit NR1 nor phosphorylation of NR1 at Ser896 were altered by BCG treatment in hippocampal tissue.
Discussion: We have shown that stimulation of the innate immune response with BCG corresponds with changes in the expression and phosphorylation state of both presynaptic and postsynaptic markers in the glutamatergic synapse in the hippocampus. Our data suggest that neuroadaptive changes in the glutamatergic system are stimulated by BCG treatment. Future studies will focus on determining the physiological relevance of these synaptic changes by looking at hippocampal dependent cognitive tasks in BCG-treated animals.
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29. Calcium Sensing Proteins in Depressive Disorder

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Background: Major depressive disorder is the most prevalent form of psychiatric disease, severely affecting 2-5% of the US population and is the major cause of suicide. Recent preclinical and clinical studies demonstrate that altered synaptic and structural plasticity play a critical role in the pathogenic mechanisms of depression; however, the precise molecular and cellular nature of events that lead to such altered plasticity remains unclear. Ca^{2+} is one of the most important molecules that play a decisive role in initiating and regulating synaptic and structural plasticity. A considerable body of evidence points to altered Ca^{2+} homeostasis and Ca^{2+} signaling in MDD. Ca^{2+} sensing proteins, such as calmodulin (CaM), and the more recently identified neuronally expressed families of Ca^{2+} binding proteins (CaBP) and neuronal Ca^{2+} sensing (NCS) proteins, which act as effector molecules to transduce Ca^{2+} signals to specific downstream events, have become a major focus of interest as molecular switches for synaptic and structural plasticity phenomena. Interestingly, each Ca^{2+} sensing protein differs: 1) in their affinity for Ca^{2+}, 2) the manner by which they respond to elevated Ca^{2+}, and 3) in their target proteins with which they interact and modulate specific neural network/pathways. In the present study we tested the hypothesis whether calcium sensing proteins play a role in depression.
Methods: To examine the status of calcium sensing proteins, mRNA levels of various eural calcium sensing proteins were determined in dorsolateral prefrontal cortex of age-, gender, and postmortem interval-matched normal controls and major depressed subjects. To examine whether the changes in expression of calcium sensing proteins are specific to depression, we examined their expression levels in dorsolateral prefrontal cortex of bipolar

and schizophrenia subjects. A total of 20 subjects in each group were included. The mRNA levels of calcium sensing proteins were determined by qPCR using TaqMan primers and probes. Geometric means of expression levels of GAPDH and beta-actin were used as endogenous control.
Results: ANCOVA, (age, postmortem interval, brain pH as covariates) followed by pairwise between-group comparisons, revealed that mRNA level of CS-1 was significantly decreased in MDD, whereas its level was increased in BPD and SCHIZ as compared to normal controls. On the other hand, mRNA levels of hippocalcin, neurocalcin δ, CaBP1, and VILIP1 were decreased only in MDD. In contrast, VILIP3 mRNA was increased in BPD. Further correlation analysis showed that mRNA levels of these eural calcium sensing proteins were not affected by age, postmortem interval, or brain pH (p values ranged between .23-.89).
Discussion: Our study demonstrates that various calcium sensing proteins are decreased in prefrontal cortex of depressed subjects. These decreases were specific to depression as schizophrenia and bipolar subjects either showed no change or showed differential regulation. Our study suggests the possibility that altered expression of calcium sensing proteins could be associated with altered neural plasticity in depressed subjects.
Disclosure: Y. Dwivedi: None. **H. Zhang:** None. **G. Pandey:** None.

30. Activity-Dependent Expression of sFRP3 Regulates Adult Hippocampal Neurogenesis and Antidepressant Actions

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Background: Major depression is one of the most common mental disorders affecting millions of people worldwide. Emerging evidence has shown that the all classes of antidepressants exhibit a significant stimulatory role on adult hippocampal neurogenesis, the process of generating mature neurons from neural progenitors. In addition, adult neurogenesisis required for some of the behavioral effects of antidepressants in rodents, suggesting that neurogenesis plays a crucial role in the mechanism of antidepressant actions. The molecular and cellular mechanisms underlying the modulation of different stages of adult neurogenesis by antidepressants are not fully understood. Here we identified secreted frizzled related protein 3 (sFRP3), a Wnt inhibitor highly expressed in the adult dentate gyrus, as a key regulator of activity-dependent modulation of neurogenesis by antidepressants in the adult brain. These findings indicate that sFRP3 serves as a key regulator that controls adult neurogenesis and antidepressant actions.
Methods: In order to evaluate the specific role of sFRP3 in adult neurogenesis and antidepressant action, we utilized multiple histological techniques, retroviral-based single cell genetic manipulations, confocal imaging, animal behaviors and animal genetic model systems.
Results: We demonstrated that deletion of the gene encoding *sfrp3* enhances several essential steps of adult hippocampal neurogenesis *in vivo*, including proliferation of neural progenitors, maturation, migration and dendritic growth of newborn dentate granule cells in the adult brain. Interestingly, stimulation of the adult hippocampal neuronal circuitry leads to a rapid decrease of *sfrp3* expression in the dentate gyrus, resulting in accelerated development of newborn granule cells *in vivo*. In addition, chronic, but not subchronic treatment with an antidepressant suppresses *sfrp3* expression in the dentate gyrus and sFRP3 null mice exhibit behavioral phenotypes mimicking wild-type mice receiving antidepressants.
Discussion: Our study identifies sFRP3 as a key regulator of not only adult neurogenesis but also antidepressant actions, and suggests a potential therapeutic target for the treatment of depression. These discoveries provide a strong body of evidence in support of the essential role of adult neurogenesis in mediating the benefits of antidepressant treatment and thus will be a foundation for further clinical studies and developing new therapeutic interventions.
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31. The FGF14:Nav Channel Complex is a New Target of the Akt/GSK3 Signaling Pathway

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Background: Mood disorders are emotionally and cognitively debilitating illnesses afflicting millions of people in the USA and potent, specific pharmacotherapies are needed. In bipolar mood disorders the increased activity of glycogen synthase kinase 3 (GSK3) correlates with neuronal hyperexcitability. Empirical evidence indicates that anticonvulsants and lithium salts that respectively minimize the activity of the voltage-gated Na + (Nav) channels and GSK3 can act synergistically in remitting clinical symptoms of bipolar disorder. This evidence suggests that mood stabilizers might converge on a common molecular target. Discovering this target will potentially provide insights into the complexity of the disease and offer an unprecedented opportunity for medication development. Previous discoveries demonstrate that the fibroblast growth factor 14 (FGF14) is a relevant component of the Nav channelosome that controls channel targeting and neuronal excitability through a direct interaction with the Nav channel intracellular C-tail. Here, we provide exciting data demonstrating that FGF14:Nav channel complex is a direct target of GSK3β and that GSK3β activity facilitates the FGF14 assembly to Nav channels, supporting a model in which increased activity of GSK3β results in hyperexcitability through modulation of the FGF14:Nav channel complex.
Methods: We have applied the split luciferase complementation assay (LCA) to detect the assembly of the FGF14:Nav1.6 channel complex real-time in living cells. Two complementary N-terminus (NLuc) and C-terminus (CLuc) fragments of luciferase were respectively fused to FGF14 and to a chimera of the CD4 transmembrane segment and the C-tail of the Nav1.6 channel. Plasmids were transiently co-expressed in HEK293 cells and interaction was detected upon d-luciferin addition as luminescence. LCA was employed to screen a library of kinase inhibitors seeking compounds that could robustly and specifically regulate the FGF14:Nav channel complex formation. Kinase inhibitors were delivered to cells 2 hours prior luminescence readings. Co-immunoprecipitation studies were conducted in HEK293 cells stably expressing full length Nav1.2 channels and transiently transfected with a plasmid vector encoding FGF14-6xmyc. Additional studies aiming at validating the effect of selective kinase inhibitors on the FGF14: Nav channel complex were conducted using quantitative immunolabeling of native FGF14 and Nav channels and single cell patch-clamp electrophysiology in primary hippocampal neurons.
Results: As a result of a kinase inhibitor screening, we show that the FGF14:Nav1.6 channel complex assembly is bi-directionally controlled by GSK3β and by the GSK3β constitutive repressor, protein kinase B (Akt). Using a combination of LCA and

co-immunoprecipitation we demonstrate that pharmacological inhibition of Akt and GSK3β increases (375 ± 24%, n = 4, p < 0.001) and prevents (22 ± 2%, n = 4, p < 0.001), respectively, the FGF14:Nav channel complex formation, whereas inhibition of GSK3β occludes the effect of Akt inhibition (51 ± 2%, n = 4, p < 0.001). Using quantitative immunofluorescence, we demonstrate that exposure of hippocampal neurons to the Akt inhibitor triciribine (30 μM, 8 hours) causes an increase in the FGF14:Nav channelosome axonal polarity compared to control, by changing the AIS/dendrite distribution of the two proteins (n = 9, p < 0.01), whereas an opposite phenotype is observed upon exposure of neurons to GSK3 XIII inhibitor (30 μM, 8 hours, n = 6, p < 0.01). Furthermore, we show that prolonged exposure of neurons to triciribine (30 μM; 24 hours) induced a statistically significant increase in maximal firing frequency (n = 5, p < 0.05), suggesting a functional correlation between excitability and the increased expression of the FGF14:Nav channel complex at the AIS induced by Akt suppression.
Discussion: While providing evidence for a novel link between GSK3β and neuronal excitability, these results offer a potential opportunity for medication development against mood disorders and other human diseases associated with hyperexcitability and dysfunction of the GSK3 signaling pathway.
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32. Optical Activation of Nucleus Accumbens Neurons Modulates Depression- and Anxiety-Like Behaviors

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Background: The nucleus accumbens (NAc) plays a crucial role in regulating mood, including maladaptive behaviors seen in depression and anxiety disorders. Recently, deep brain stimulation (DBS) of the nucleus accumbens was shown to alleviate depression and anxiety symptoms in patients suffering from treatment resistant depression.
Methods: We employ optogenetic technologies to apply repetitive (5 days) high frequency (130 Hz) and low frequency (10 Hz) stimulation to NAc neurons, using adeno-associated viruses (AAVs) expressing Channelrhopsin-2 (ChR2), in animals exhibiting social avoidance (a depression-like behavior) after chronic social defeat stress. Additionally, we optogenetically activate specific NAc neuronal subtypes with a low frequency stimulation (10 Hz) during acute mood-related behaviors including tail suspension test (TST–a form of stress) and elevated plus maze (EPM–a measure of anxiety).
Results: Repetitive high frequency, but not low frequency, ChR2 activation of NAc neurons reversed the social avoidance seen after chronic social defeat stress. Furthermore, low frequency stimulation of dopamine receptor 1 (D1)-containing NAc projection neurons during acute mood-related behaviors decreased time spent immobile during the TST and increased time in the open arms in the EPM.
Discussion: These results provide insight into the mechanism of DBS stimulation in the NAc, since directly activating NAc neurons repetitively with high frequency stimulation can alleviate depression symptoms displayed after chronic social defeat stress. Additionally, we show that activation of D1-containing NAc neurons is important for mediating acute mood-related behaviors, since activation of these neurons has antidepressant- and anxiolytic-like effects.
Disclosure: S. Zaman: None. **P. Kennedy:** None. **D. Dietz:** None. **E. Nestler:** None. **M. Lobo:** None.

33. Gene Expression from Lymphoblastoid Cell Lines and Brain Tissue in Bipolar Disorder: Convergent and Divergent Patterns

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Background: The hereditary basis of bipolar disorder (BD) has been established through family, twin, and adoption studies. Previous and current genetic mapping and expression analyses are consistent with a polygenic inheritance model; numerous risk loci associated with BD have been reported, each with a modest risk (OR 1.1–1.3). In a complementary approach, a number of studies aimed at identifying transcripts differentially expressed in post-mortem brains or in blood cells from affected individuals compared with healthy individuals have been published. We hypothesized 1) there are gene expression changes in peripheral tissue common with those in CNS tissue and 2) the effects of medical treatment are mediated through gene expression changes.
Methods: We studied 2 independent and unrelated sources of RNA: 1) lymphoblastoid cell lines (LCL) from 49 sibling pairs discordant for BD, and 2) total RNA extracted from 0.2 g of frozen brain tissue (premotor cortex) from Bipolar and control subjects from the Stanley Brain collection. For brain samples, the samples were categorized into: taking typical antipsychotics at the time of death (n = 7), not taking typical antipsychotics at the time of death, n = 7), and controls (C, n = 12), to fit the multi-class algorithms of SAM. Expression analyses were performed with the Sentrix HumanRef8_V2 BeadChip microarrays (Illumina, CA) on LCLs, while the GeneChip U133 plus 2.0 (U133P2) (Affymetrix), Santa Clara, CA) was used for expression analysis of RNAs from postmortem brains. The expression pattern of 12 randomly selected genes was validated with RT-PCR. For analysis of the HumRefseq8-V2 arrays, the BeadStudio software was used to process raw data for quality control and to perform data normalization using robust multi-array average (RMA) algorithms. For analysis of the U133P2 arrays, the raw data (CEL files) were processed using the Bioconductor package "Affy" for R. The Significant Analysis of Microarrays (SAM) software package for Microsoft Office Excel was used for statistical analysis and identification of significantly altered genes. The expression analysis systematic explorer (EASE) software was employed for Gene Ontology (GO) term enrichment and pathway analysis.
Results: From the LCLs, 582 transcripts (545 unique genes) were detected that significantly differed in abundance between affected and unaffected siblings using a SAM q value (equivalent to false discovery rate, FDR) at ⩽0.1. The expression profiles of the antipsychotic exposed group were very similar to that of controls, and most significant changes were between pooled control and antipsychotic treated groups versus the group unexposed to antipsychotics. SAM two-class algorithms for differential expression analysis identified 2191 unique genes represented by 2818 probe sets that were differentially expressed in the samples unexposed to antipsychotics compared to the pooled group (q ⩽ 0.005). Between the 582 transcripts in the LCL list and the 2191 unique genes in the brain list, gene expression profiles identified in LCLs from sibling pairs discordant for BD and postmortem brains from affected individuals and unrelated controls identified expression of 103 genes was commonly changed in both (p < 0.02).
Discussion: These findings suggest that the study of peripheral gene expression patterns may be informative in BP disorder. There were common gene expression changes in independent and unrelated samples. There are generalized treatment affects on gene expression in brain. RNAs from brain tissue from BP individuals were analyzed taking into consideration the exposure to typical antipsychotics and compared with normal controls, there were significant differences in gene expression between groups. Functional gene ontology (GO) term enrichment analysis of genes

from the LCL list are enriched for 19 nominal significant GO terms, one category, nucleosome assembly, remained significant after correction for multiple tests. From the brain list, significant GO terms (Bonferroni $P < 0.05$) of biological processes were protein metabolic process, transport, localization, post-translational protein modification, protein folding, and synaptic transmission; significant GO terms of molecular function identify protein and nucleotide categories including ATP binding, ligase, and catalytic activities.
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34. Circulating Abeta40 Negatively Influences Plasma BDNF Levels

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Background: Reductions in brain-derived neurotrophic factor (BDNF) have been implicated in the pathophysiology of depression. Nevertheless, the factors influencing central and peripheral BDNF levels are still poorly understood. Cerebral microvascular endothelial cells are known to be a major source of BDNF within the brain. Exposure of these cells to amyloid beta (Abeta), which may play a role in the pathophysiology of late-life depression, results in cell death or injury with significant reductions in BDNF secretion. Moreover, in rodents, infusion of Abeta40 into the carotid artery resulted in a disruption of endothelial cells, which was not observed with Abeta42 infusion. Therefore, we hypothesized that concentrations of plasma Abeta40, but not Abeta42, would have a negative effect on plasma BDNF levels.
Methods: We examined BDNF and Abeta levels in plasma via immunoblotting and ELISA assays, respectively, from 88 subjects with intact cognition (no dementia and a Mini-Mental State Exam score of at least 28) and no gross MRI abnormalities other than white matter hyperintensities. As these subjects were originally recruited for a study on major depressive disorder (MDD), 45 had MDD and 43 were age-matched controls.
Results: Consistent with our prediction, Abeta40 levels were inversely correlated with BDNF concentrations ($p < .001$), whereas Abeta42 levels were independent of BDNF expression ($p = .231$). This pattern was similar when MDD and control subjects were analyzed separately.
Discussion: Our results are consistent with the hypothesis that cerebral endothelial cells are a contributing source of peripheral BDNF and that their disruption by circulating Abeta40 results in reduction in BDNF. However, these preliminary findings need confirmation, and the mechanisms for our observation, including Abeta40-induced cerebral endothelial cell dysfunction, will have to be clarified.
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35. Incomplete Coverage of Blood Vessels in Orbitofrontal Cortex by Astrocytic End-feet in Major Depressive Disorder

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Background: Depression and cardiovascular disease are often comorbid. In the frontal lobe in major depressive disorder (MDD), physiological studies report impairment of endothelial functions and neuroimaging studies demonstrate alterered blood vessel size and density. To date, however, no studies have been conducted on vascular morphology at the microscopic and molecular level in depression.Our previous postmortem studies analyzing glial fibrillary acidic protein, GFAP-immunoreactive astrocytes in MDD revealed a marked deficit in the density of these cells and in the area fraction covered by their immunoreactive processes. Since astrocyte processes enwrap the blood-brain barrier, we hypothesized that expression of GFAP in orbitofrontal cortex (ORB) is reduced in depressed subjects, and is also accompanied by decreased coverage of blood vessels by astrocytic endfeet.
Methods: Postmortem brain tissue from 11 pairs of MDD and control subjects was used. Frozen sections from ORB were collected to measure the co-localization of markers for blood vessels (collagen IV) and astrocytes (GFAP) by using double immunohistochemistry and confocal microscopy. Co-localization of collagen and GFAP was quantified by measuring the area of coverage of vessels by astrocytic endfeet. Expression of mRNA for GFAP was measured in punches of ORB by using real time polymerase chain reaction (RT PCR) whereas GFAP protein level was measured using Western blots.
Results: There was a significant 51% reduction (unpaired t-test: $t = 2.18$, $df = 12$, $p = 0.049$) in the fraction of vessel area covered by GFAP immunoreactive processes in the ORB of young (< 60 years of age, 7 pairs) subjects with MDD. Expression of GFAP mRNA was also reduced by 55% ($p = 0.06$) and level of GFAP protein was reduced by 50% (ANCOVA: $F\ (3,\ 42) = 3.759$, $p = 0.018$) In contrast, older (> 60, 4 pairs) subjects with MDD showed a trend for an increase (unpaired t-test: $t = 2.18$, $df = 6$, $p = 0.072$) in the fraction of vessel area covered by GFAP.
Discussion: Significantly reduced coverage of blood vessels by astrocytic processes in MDD may contribute to the vascular pathology reported by postmortem and structural neuroimaging studies in the ORB. Incomplete coverage may also contribute to altered glucose metabolism detected in neuroimaging in this cortical area. Supported by P20RR17701.
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36. Epigenetic Modifications in Postmortem Frontal Cortex from Bipolar Disorder and Alzheimer's Disease Patients

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Background: Bipolar disorder (BD) and Alzheimer's disease (AD) are chronic, disabling, progressive, neuropsychiatric illnesses with some overlapping molecular features. Epigenetic mechanisms of gene expression have been implicated in the pathophysiology of brain disorders. Recently, we reported upregulated neuroinflammatory and arachidonic acid (AA) markers plus loss of brain derived neurotrophic factor (BDNF) and synaptic markers (synaptophysin and drebrin) in postmortem frontal cortices from BD and AD patients.
Methods: We hypothesized that changes in AA cascade, neurotrophic and synaptic markers are related to changes in their promoter site CpG methylation. To test this hypothesis, we measured global DNA methylation and gene specific CpG methylation as well as histone acetylation, phosphorylation and methylation in postmortem frontal cortex of BD ($n = 10$) and AD ($n = 10$) patients along with age matched controls ($n = 20$).
Results: BD and AD brains showed global DNA hypermethylation. The CpG islands in the promoter regions of cyclooxygenase-2 (COX-2) and BDNF genes were hypo and hyper-methylated in AD

and BD patients compared to controls, respectively. Further, CpG islands in the promoter regions of GAD1 and drebrin both were hypo and hyper-methylated in BD but not in AD patients. BD and AD brains showed an increased global histone phosphorylation state. Epigenetic modifications for COX-2, BDNF and drebrin in BD and AD were consistent with corresponding inverse changes in their mRNA and protein levels. However, there were no significant changes in CpG methylation in promoter regions for synaptophysin, p450 epoxygenase or DNA methyl transferase 3A enzymes in both illnesses.

Discussion: This study demonstrates overlapping changes in epigenetic modification of COX-2 and BDNF genes in BD and AD patients. Furthermore, there are additional epigenetic modifications that are associated with BD at the GAD1 and drebrin promoter regions. These epigenetic modifications may contribute to disease pathophysiologies and could be therapeutic targets for future drug development.

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37. Optogenetic Control of Mesolimbic Dopamine Neural Activity Recapitulates the Anxiety-Related Phenotype of the Clock-Δ19 Mouse Model of Mania

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Background: There is accumulating evidence to suggest a role for altered circadian rhythms in the pathophysiology of bipolar disorder. Indeed, previous work in our lab has shown that mice with a point mutation in the circadian gene, *Clock* (*Clock*-Δ19), display manic-like behaviors that are attenuated by lithium treatment. Evidence from our lab suggests that altered dopaminergic (DA) activity in the mesolimbic system, a region implicated in reward and mood regulation, may underlie components of this behavioral phenotype. However, a direct causal link between long-term disruption of mesolimbic DA activity and manic-related behavior has yet to be established.

Methods: Alterations in mesolimbic dopamine activity were examined at a molecular level by assessing tyrosine hydroxylase (TH) mRNA and protein expression and at a systems level through *in vivo* electrophysiological recordings. In order to directly determine the role of altered DA neural activity on manic-related behaviors, we employed optogenetics and introduced a unique microbial channelrhodopsin (step-function opsin) into the ventral tegmental area (VTA) of TH::Cre mice to optically control dopamine neural activity over an extended period of time (1hr/day for one week). A behavioral battery was performed to determine whether increased DA activity recapitulated features of the *Clock*-Δ19 mouse manic-like phenotype.

Results: *Clock*-Δ19 mice displayed an up-regulation of VTA TH mRNA expression and protein levels that were accompanied by an increase in bursting and firing of VTA dopamine neurons. To determine to what extent this molecular phenotype drives manic-like behaviors, TH::Cre mice underwent chronic optical stimulation of VTA neurons to increase DA neural activity. Chronic depolarization of DA neurons in the VTA recapitulated components of the *Clock*-Δ19 mouse manic-like behavioral phenotype as indicated by increased risk-taking and reduced anxiety-like behaviors, with no alteration in depressive-related behaviors.

Discussion: This is the first report on the ability of long-term optical manipulation of neural activity to alter baseline rodent behavior. Using this approach, we demonstrated a direct role for increased VTA dopamine activity in mediating specific features of manic-related behavior. These results provide insight into the biological underpinnings of mood disorders and facilitate future study on the impact of chronic stimulation of multiple neurotransmitter systems on complex behavioral phenotypes.

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38. Peripheral Biomarkers in New Onset of Major Depressive Disorder in Midlife Women: The Harvard Study of Moods and Cycles

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Background: The transition to menopause and early postmenopausal years have been associated with increased risk for developing depressive episodes (new onset or recurrent). Recent studies suggest the existence of peripheral markers in mood disorders (e.g., oxidative damage, inflammatory markers, neurotrophic factors) that may be relevant to the understanding of disease pathophysiology, activity and progression. No published studies to date have explored the presence of/changes in peripheral biomarkers in subjects who did and did not develop new onset of Major Depressive Disorder (MDD). In this study, we examine the presence/emergence of peripheral biomarkers for new onset of MDD in a longitudinal, prospective cohort study – the Harvard Study of Moods and Cycles (HSMC).

Methods: Euthymic, never-depressed premenopausal women (N = 460, aged 36-45 years) were enrolled into the HSMC and assessed prospectively (every 6 months) to examine the association between reproductive/endocrine menstrual changes (i.e., the transition to menopause) and the onset of first lifetime episode of MDD. For this analyzes, we included subjects who developed first-onset of MDD during the first 3 years of follow up (N = 29) and matched controls (i.e., women who remained euthymic, N = 29). The following potential peripheral biological markers were examined in blood/serum samples (1) *Markers of oxidative stress:* Thiobarbituric acid reactive species (TBARS), protein carbonyl content, total reactive antioxidant potential (TRAP); (2) *Neurotrophic factors:* Brain-derived neurotrophic factor (BDNF), and (3) *Inflammatory markers*: interleukin-6 (IL-6), interleukin-10 (IL-10). Analyzes were performed with samples collected at 3 different time points, with matching time for controls: at study entry, at the last visit prior to the diagnosis of MDD (time point 1) and at the time point when MDD was diagnosed (time point 2). Blood/serum samples were stored at −80°C. Experimental procedures included: malondialdehyde and carbonyl content, IL-6, IL-10 and BDNF levels, all analyzed with high-sensitive ELISA kits; oxidation/reduction in thiol groups, and total non-enzymatic antioxidant potential (TRAP assay). Total protein content (Lowry) and uric acid content were also quantified. All data were analyzed

using t test and two-way ANOVA with repeated measures. Differences were considered significant at $P < 0.05$.
Results: First-onset MDD subjects (mean 41.2 ± 0.47 years) and controls (mean age $= 41.1 \pm 0.47$ years) were not on any psychotropic medications at the time of study assessments and had no significant differences regarding demographics, reproductive staging, smoking or BMI. Comparative analyzes of samples collected from first-onset MDD subjects and matched controls at baseline, time point 1 (pre-diagnosis of MDD; 11.8 ± 2.05 and 12.6 ± 2.03 months, respectively) and time point 2 (diagnosis of MDD; 18.6 ± 2.09 and 18.6 ± 2.09 months) did not reveal significant differences with respect to any of the peripheral biomarkers investigated (TBARS, TRAP, Carbonyl, IL-6, IL-10, and BDNF; $p > 0.05$ for all comparisons). Moreover, changes in all these biomarkers across the time points were similar between the 2 groups.
Discussion: In this study, changes in oxidative damage, inflammatory response or BDNF levels were neither predictive not indicative of first-onset of major depression. Future studies should clarify whether the absence of peripheral biomarkers could be attributed to this particular illness (MDD), the short duration of exposure to the illness, (i.e., recently emerged, newly diagnosed MDD) or to the specific sub-population examined (midlife women).
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39. Influence of Major Depressive Disorder on the Volume and Number of Neurons, Glia, and Perivascular Cells in the Basolateral Amygdala: A Postmortem Stereological Study

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Background: The basolateral amygdala (BLA) is implicated in the pathophysiology of major depressive disorder (MDD). Functional imaging studies consistently report heightened amygdala reactivity and disturbed amygdala-frontal connectivity in depressed patients; and postmortem studies have shown alterations in measures such as BLA neurotransmitter receptors and amygdalar glial cell density. Imaging studies of amygdala volume in depression have however produced a very mixed literature, and no postmortem studies to date have examined the volume or the total number of cells in the BLA of depressed subjects. Here, we undertook a stereological analysis of the volume and total number of neurons, glia and perivascular (pericytes and endothelial) cells in the BLA in subjects with MDD and psychiatrically healthy controls.
Methods: Postmortem tissues from 13 subjects with unremitted MDD and 10 controls were coded, fixed in formalin, and embedded in celloidin. Forty-micron thick sections were taken throughout the rostral-caudal extent of the BLA, and every tenth section Nissl-stained for microscopic analysis. The Cavalieri principle and the optical fractionator were used for unbiased stereological estimation of volume and cell numbers respectively in the lateral, basal, and accessory basal nuclei.
Results: There were no significant effects of diagnosis on amygdala volume; however among depressed subjects, duration of depression was significantly negatively correlated with total BLA volume ($p = .021$). Although not statistically significant, there was a trend for lateral nucleus volume to be increased in females with MDD ($p = 0.065$) when compared to female controls; no such difference was seen in males. In addition, there was a strong trend ($p = .0501$) for total number of pericytes and endothelial cells to be increased in the BLA in the MDD group.
Discussion: Several hypotheses have been advanced to explain the mixed literature on amygdala volume in depression, including the idea that amygdala volume is increased in the initial stages of depression only (Frodl *et al.*, 2003). Our finding that BLA volume is negatively correlated with depression duration tends to support the hypothesis that amygdalar volumetric changes in depression are duration-dependent. Although not statistically significant, the trend for lateral amygdala volume to be increased in depressed females, but not males, may represent another source of variability in the literature, and is consistent with studies suggesting there are sexually dimorphic amygdala responses to depression and anxiety (Hastings *et al.*, 2004; van der Plas *et al.*, 2010; van Elst *et al.*, 2000). The strong trend for an increase in amygdala perivascular cells in depression may be related to the commonly observed metabolic alterations in the amygdala of depressed subjects.
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40. Selective Optogenetic Stimulation of Parvalbumin-Positive Basal Forebrain Neurons Reliably entrains Cortical Gamma Oscillations and Promotes Wakefulness

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Background: The basal forebrain (BF) plays a crucial role in the modulation of cortical activity across sleep-wake cycles via cortically projecting cholinergic and non-cholinergic neurons. One of the more important facets of cortical activation is the presence of gamma band (40 Hz) activation, known to be important in feature binding and to be impaired in schizophrenia. However, little is known about the role of the BF in this important feature. Among non-cholinergic neurons in BF, parvalbumin (PARV)-containing, gamma-aminobutyric acid (GABA)ergic neurons are one of the important components. They project to GABAergic PARV + cortical neurons and their firing rates increase during the electroencephalographic (EEG) low-voltage fast activity characteristic of waking. However, their precise contribution to cortical activation and sleep-wake regulation is not well understood. Therefore we sought to selectively express a light-activated opsin (i.e. channelrhodopsin2, ChR2) in PARV-positive neurons within BF to enable their selective activation using optogenetic stimulation, and to determine the effect on the EEG and sleep-wake behavior.
Methods: To target channelrhodopsins selectively to BF PARV neurons, adeno-associated viral vectors with double-floxed Channelrhodopsin2 (ChR2)-eYFP were injected stereotactically into the BF of transgenic mice expressing Cre recombinase under the

control of the PARV promoter (PARV-Cre mice). Posthoc immunohistochemistry was done for histological confirmation of selective expression. To evaluate the physiological effect of activation of BF PARV neurons, optical stimulation (laser light, 473 nm, 10 ms pulse width, various frequencies from 2 to 60 Hz) was delivered through an optical fiber inserted into a guide cannula targeting the BF. The effect on the sleep-wake cycle was investigated by comparing one hour of baseline EEG with that of same time of day of one hour of optical stimulation (40 Hz frequency, 5 s train duration, every 60 s).
Results: Immunohistochemistry, performed at least two weeks after injection of the virus, confirmed high levels of double labeling of ChR2-eYFP (green) and PARV (red) indicating selective expression of ChR2-eYFP in BF PARV neurons. BF entrainment of the cortical EEG was particularly dramatic when the BF stimulation was at the gamma oscillation frequency (40 Hz). Notably, this entrainment could be reproducibly elicited over the course of an hour of stimulation; each and every train of 40 Hz BF stimulation was reliably followed by cortical 40 Hz activity. 20 Hz stimulation elicited a clear 40 Hz harmonic. The sleep-wake behavior was altered by optical stimulation, increasing wakefulness from 9.2 % to 45.2 % and decreasing NREM sleep from 75.3 % to 43.5 %, excluding the 5 s of stimulation.
Discussion: We believe this BF PARV-specific elicitation of cortical gamma oscillation has not been previously reported, and may represent an important but unsuspected feature of BF activation and of generation of cortical gamma oscillations. We conclude that optogenetic stimulation of PARV-positive BF neurons entrains cortical rhythms, particularly those in the gamma range, and enhances wakefulness. A role of abnormalities of BF in the abnormalities of cortical gamma oscillations in schizophrenia appears to be an intriguing, but as yet unexplored, possibility raised by these findings.
Disclosure: T. Kim: None. **J. McKenna:** None. **J. McNally:** None. **K. Deisseroth:** None. **R. Strecker:** None. **R. Brown:** None. **R. Basheer:** None. **R. McCarley:** Part 1: Consultant to Merck, Janssen, Takeda, Sunovion.

41. The Power of Expectation Bias

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Background: Researchers have identified several sources of bias in clinical trials that may lead to increased placebo response and decreased signal detection. One such bias, expectation bias, is of particular concern in CNS clinical trials due to the subjective nature of many (including primary) outcomes. Expectation bias occurs when an individual's expectations about an outcome influence one's perceptions of one's own or others' behavior. In psychiatric clinical trials, both subjects and raters may enter the trial with expectations for the outcome. Subject expectation bias may occur when subjects themselves expect to get better or report improvement to please the rater. Rater expectation bias may occur when raters expect that subjects will improve (or fail to improve) over the course of the trial. Finally, in traditional trial designs, rater and subject expectations may interact to create a therapeutic alliance. Any or all of these may result in increased placebo response and decreased drug-placebo separation. Double blind studies are designed to control for expectation bias by blinding the subject and the clinical rater to whether the subject is taking placebo or drug. However, other factors to which subjects and raters are typically unblinded, such as inclusion/exclusion criteria, adverse events and visit sequence (i.e., duration of treatment) may also affect placebo response.
Methods: We reviewed eight published studies that illustrate the problems of subject and rater expectation bias across several therapeutic areas including Major Depressive Disorder (MDD), Generalized Anxiety Disorder (GAD), psychosis, Parkinson's disease and dementia.
Results: Results of three studies of subject expectation bias suggest that subject expectations regarding their likelihood of receiving active treatment can increase placebo response and affect study outcome. Results of studies examining rater expectation bias suggest that rater expectations can affect subject diagnosis and also decrease inter-rater reliability when subjects do not behave according to pre-conceived expectations. Finally, studies of the interaction of rater and subject expectation find that placebo response increases linearly with the number of follow-up visits, and that having a different rater for baseline, endpoint, and sequential visits may decrease placebo response.
Discussion: Expectation bias may have an effect when subjects' reports and raters' scores are influenced by the expectation of improvement. The studies reviewed here suggest that patient expectations, rater expectations, and rater-patient interactions can increase placebo response and decrease signal detection. Taken together, these results suggest that using raters who are blinded to study protocol details, including inclusion/exclusion criteria and study visit number, may yield better signal detection and lower placebo response. Blinding to protocol details and study visit number eliminates the possibility that clinical ratings will be affected by an expectation of improvement as treatment progresses. Further, using a different rater at baseline, endpoint and for consecutive visitscontrols for the possibility of a relationship (sometimes referred to as relationship bias) between rater and subject that could influence ratings.
Disclosure: J. Williams: Part 1: MedAvante, Inc., Part 2: MedAvante, Inc., Part 3: MedAvante, Inc., Part 5: MedAvante, Inc. **D. Popp:** Part 1: MedAvante, Inc., Part 2: MedAvante, Inc., Part 3: MedAvante, Inc., Part 5: MedAvante, Inc. **K. Kobak:** Part 1: MedAvante, Inc., Part 2: MedAvante, Inc., Part 3: MedAvante, Inc. **M. Detke:** Part 1: MedAvante, Inc.; Eli Lilly, Inc.; Sonkei, Inc., Part 2: MedAvante, Inc.; Eli Lilly, Inc., Part 3: MedAvante, Inc.; Eli Lilly, Inc., Part 5: MedAvante, Inc.

42. A Pilot Study Evaluating the Cognitive Effects of Rimonabant in People with Schizophrenia

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Background: Rimonabant is a cannabinoid 1 (CB1) antagonist, which is effective in treating obesity in the general population. Human and animal studies suggest that rimonabant may also have significant effects on cognitive function. The current study was designed to examine the effects of add-on rimonabant on neurocognitive test performance in people with schizophrenia.
Methods: We conducted a 16-week, double-blind, placebo-controlled study of rimonabant (20 mg/day) in people with DSM-IV schizophrenia or schizoaffective disorder, who were clinically stable on second generation antipsychotics. Participants had a BMI $^{3}27\,kg/m^{2}$ with hyperlipidemia or BMI $^{3}30\,kg/m^{2}$, in the absence of current substance abuse/dependence (except nicotine), more than weekly cannabis use, or recent depressive symptoms/suicidality. An exercise and dietary counseling group was offered weekly. We used the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), the Iowa Gambling Task (IGT), the N-Back task, and a probabilistic learning task (PL) to examine the effects of rimonabant on cognition. Target enrollment was 60; the trial was terminated early due to withdrawal of rimonabant from the European market.
Results: Fifteen participants were randomized (7 rimonabant, 8 placebo). Fourteen participants had baseline and end of study

neuropsychological data (7 in each group). There was a significant treatment effect for the RBANS total score, with the placebo group exhibiting a small improvement and the rimonabant group exhibiting a small worsening on this measure (estimated difference [mean ± s.e.): −7.7 ± 3.5; p = 0.048; effect size = 0.63). There were no significant group differences for any of the RBANS scales. There were no significant group differences on the IGT, or the N-Back task. In contrast, rimonabant was associated with significant improvement on the PL task, which were driven by improvement on the 80:20 (effect size: 1.03; p = 0.11) and 70:30 (effect size = 1.07; p = 0.06) reward probability conditions. There was also a significant rimonabant effect on positive feedback, as measured by staying with rewarded choices (effect size = 1.88, p = 0.009).

Discussion: Rimonabant did not improve global cognitive functioning, however a specific deficit in learning based on positive feedback did improve with rimonabant treatment. More research is required to determine how the endocannabinoid system relates to psychopathology and neurocognitive impairments in schizophrenia and whether alteration of this system can lead to novel therapeutic treatments. Supported by the NIMH 1 R34 MH077839 (P.I.: Robert W. Buchanan), NIDA Contract N01DA59909 (P.I.: Deanna L. Kelly), and the Intramural Research Program, NIH, NIDA.

Disclosure: R. Buchanan: Part 1: DSMB member for Cephalon, Otsuka, and Pfizer; Consultant for Abbott, Amgen, Cypress Bioscience, Glaxo-Smith-Kline, Merck, Sanofi-Aventis, Solvay, Takeda, and Wyeth; and Advisory Board member for Abbott, Amgen, Astellas, Astra-Zeneca, Merck, Pfizer, Roche, Solvay Pharmaceuticals, Inc., Wyeth, Part 4: Psychogenics. **D. Boggs:** None. **R. Conley:** Part 5: Eli Lilly and Company, Indianapolis, Indiana. **D. Gorelick:** None. **R. McMahon:** Part 4: Psychogenics. **J. Gold:** Part 1: Consultant for Solvay, Glaxo Smith Kline, Pfizer, Merck, Astra Zenaca, and Roche. **J. Waltz:** None. **M. Huestis:** Part 4: A NIH Cooperative Research and Development Agreement with Sanofi-aventis. **D. Kelly:** None.

43. Movement Disorder Trajectories and Treatment Outcomes in a 1-Year Study of Patients with Schizophrenia

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Background: Movement disorders have a long history of association with antipsychotic treatment. Antagonism of dopamine D2 receptors resolves psychosis, but at the same time induces extrapyramidal side effects (EPSs), one of the most troubling side effects of antipsychotic treatment. Compared with the first generation antipsychotics (FGAs), the second generation antipsychotics (SGAs) are associated with a reduced risk of inducing acute EPS at recommended dose ranges. EPS liability tends to vary among the SGAs as well as by target dose, rapidity of dose escalation, and patient's vulnerability to EPS. It is currently unknown whether patients with schizophrenia who differ on their EPS or movement disorder trajectories over time (e.g., increased, decreased, or unchanged severity), also differ in their clinical and functional outcomes. This study aimed to identify subgroups of patients who differ on their movement disorder trajectories over a 1-year period and compare their clinical and functional outcomes.

Methods: For this post-hoc analysis, we used data of a randomized, open-label, 1-year study of patients with schizophrenia who were treated in the U.S. with FGAs or SGAs in usual clinical care settings (F1D-US-HGGD). The study assessed EPS and Tardive Dyskinesia using the Abnormal Involuntary Movement scale, the Simpson-Angus scale, the Barnes Akathisia Rating scale, spontaneously reported movement disorder events, and use of antiparkinsonian medications. These 5 measures were incorporated into a single Movement Disorder Index (MDI), which was calculated for each subject at every study visit, with higher scores indicating more severe movement disorder. The MDI was used to identify subgroups of patients who differed in their MDI trajectories over the 1-year study. These patient subgroups were then compared on various treatment outcomes, including time to study dropout, symptom severity per Positive and Negative Syndrome Scale (PANSS) total and subscale scores, level of mental and physical functioning per SF-36, and use of hospitalization and emergency services per Resource Utilization Form. The PANSS and the SF-36 scores were analyzed using Mixed Model Repeated Measures (MMRM) models adjusted for baseline values. Chi-square test was used to compare patient subgroups on hospitalization and emergency service use. Log-rank test was used for time to study dropout. Missing values on individual MDI score were handled by last observation carried forward (LOCF).

Results: For the overall population, the mean MDI score decreased from 0.97 at baseline to 0.65 at 1-year endpoint. Three trajectories of the MDI were indentified: an increase in MDI over the 1-year study was evident for 15% of the patients (n = 94); a decrease in MDI was observed for 33% (n = 207), and 52% evidenced no change in MDI (n = 330). Although there was no statistically significant difference in the time to study dropout among the 3 subgroups, the patients who evidenced an increase in MDI over time were found to have significantly poorer clinical and functional outcomes on several measures compared with the other 2 subgroups (p values < .05). Patients who had an increase in MDI had poorer symptom improvement per PANSS total score (mean changes of -10.8, -18.0, and -16.7 for the patients who had an increase, no change, and a decrease, respectively). Patients who had an increase in MDI also had poorer symptom improvement per PANSS subscale scores (except for the Impulsive/Hostility subscale score),and less improvement on the SF-36 physical functioning component score. Patients who had an increase in MDI also had a higher rate of hospitalization (37%, 19%, and 21% for the patients who had an increase, no change, and a decrease, respectively).

Discussion: This post-hoc analysis found that patients with schizophrenia who were treated with antipsychotics and experienced worsening of their movement disorder severity level over up to a year, as measured by a Movement Disorder Index (MDI), also evidence poorer clinical and functional outcomes, including higher rates of hospitalization and emergency services. Sensitivity to antipsychotic-induced movement disorder may be a clinical marker for poor treatment prognosis. The link between patients' movement disorder liability and treatment outcomes will require further study, along with replication of the findings with the promising new Movement Disorder Index.

Disclosure: L. Chen: Part 5: Eli Lilly and Company. **H. Ascher-Svanum:** Part 1: Full time employee of Eli Lilly and Company, the sponsor of the study, Part 2: A minor stockholder in Eli Lilly and Company, the sponsor of the study, Part 3: A minor stockholder in Eli Lilly and Company, the sponsor of the study, Part 5: Eli Lilly and Company. **A. Lawson:** Part 1: Employee and shareholder of Eli Lilly and Company, Part 2: Employee and shareholder of Eli Lilly and Company, Part 3: Employee and shareholder of Eli Lilly and Company, Part 5: Eli Lilly and Company. **V. Stauffer:** Part 1: Employee Eli Lilly and Company, Part 5: Employee Eli Lilly and Company. **A. Nyhuis:** Part 1: I am a full-time employee of Eli Lilly., Part 2: See above, Part 3: See above, Part 5: I am a full-time employee of Eli Lilly. **V. Haynes:** Part 2: Employee of i3 Research, employee of Eli Lilly, Part 5: Employee of i3 Research, employee of Eli Lilly. **K. Schuh:** Part 1: I am a full-time employee of Eli Lilly and Company and minor shareholder. My wife, Leslie Schuh, has received

a grant to serve as a principal investigator for a clinical trial funded by Eli Lilly and Company, Part 2: I am a full-time employee of Eli Lilly and Company and a minor shareholder. My wife, Leslie Schuh, has received a grant to serve as a principal investigator for a clinical trial funded by Eli Lilly and Company, Part 3: I am a full-time employee of Eli Lilly and Company and a minor shareholder, Part 4: My wife, Leslie Schuh, has received a grant to serve as a principal investigator for a clinical trial funded by Eli Lilly and Company. She also received grants from Ethicon and Allergan to support a scientific conference at St. Vincent Hospital in Carmel, Indiana, Part 5: Eli Lilly and Company. **B. Kinon:** Part 1: Shareholder in Eli Lilly and Company, Part 2: Employee of Eli LIlly and Company Shareholder in Eli Lilly and Company, Part 3: Employee of Eli LIlly and Company Shareholder in Eli Lilly and Company, Part 4: None, Part 5: Employee of Eli LIlly and Company.

44. Does Acute Oxytocin Administration Enhance Social Cognition in Individuals with Schizophrenia?

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Background: Individuals with schizophrenia frequently have deficits in social cognition (e.g., theory of mind, social perception, attributional bias, and emotional processing). Social cognition has been found to be critical in predicting multiple aspects of community functioning. However, treatments for these deficits are inadequate; psychosocial treatments are limited in their effectiveness and not widely available, and there are no current pharmacological treatments. The oxytocin system, given its important roles in social behavior and empathy, has garnered much recent attention in research on schizophrenia and other disorders involving social cognitive deficits. In this study, we test the hypothesis that acute administration of intranasal oxytocin will improve social cognitive function in individuals with schizophrenia.
Methods: Twenty-four male veterans between the ages of 18 and 55 who meet the DSM-IV-TR criteria for schizophrenia are being enrolled in this trial. At their initial visits, baseline neuropsychological assessments of "high level" (The Awareness of Social Inference Test: Social Inference – Enriched and Emotional Perspective Taking Task) and "low level" (Half-Profile of Nonverbal Sensitivity and facial affect recognition task) social cognition are being performed, as are baseline assessments of schizophrenia symptom severity (Positive and Negative Syndrome Scale). One week later, patients are randomized to receive a single dose of 40 IU intranasal oxytocin or placebo in a double-blind fashion, and the neuropsychological assessment battery and schizophrenia symptom severity assessment are repeated. The change in social cognitive function between the baseline and treatment visits will be compared between placebo- and oxytocin-treated patients.
Results: Ten individuals have thus far been enrolled in this trial, with 8 having completed the entire protocol. The mean age of these participants was 49. The mean number of years since initially experiencing psychotic symptoms was 31. Two participants were receiving typical antipsychotics, 6 were receiving atypical antipsychotics, and 2 were receiving a combination. The mean MIRECC Social GAF was 58. The study will be unblinded after 24 subjects have completed the protocol, and complete analysis will subsequently occur. At the current rate of enrollment, all subjects will have completed this study by the end of October, 2011.
Discussion: The oxytocin system is an attractive target for improving social cognition in individuals with schizophrenia, as evidenced by previous studies from other investigators. This study, when completed, will add to our current knowledge base by determining whether a single dose of intranasal oxytocin improves specific domains of social cognition.
Disclosure: M. Davis: None. **J. Lee:** None. **M. Green:** Part 1: Consultant - Abbott Laboratories, Amgen, Cypress, Lundbeck, and Teva. Speaker - Otsuka and Sunovion. **S. Marder:** Part 1: Consultation and Advisory Boards: Amgen, Astellas, Abbott, Roche, Targacept, Otsuka, Pfizer, Shire, Lundbeck, Part 2: Stockholder: Med Avante, Part 4: Research Support: Glaxo Smith Kline, Novartis, Sunovion.

45. An Intervention to Test the Alpha7 Nicotinic Receptor Model in Schizophrenia: CDP-choline, a Cholinergic Agonist, and Galantamine, a Positive Allosteric Modulator

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Background: Deficient transduction of the cholinergic signal by the α_7 nicotinic acetylcholine receptor (α_7 nAChR) is implicated in the pathophysiology of schizophrenia. Further, the α_7 nAChR may be a pharmacotherapeutic target in negative symptom schizophrenia, using α_7 nAChR agonists. Choline is a directly acting agonist; CDP-choline is a dietary source of choline. However, the sensitivity of the receptor can alter upon tonic exposure to an agonist. Galantamine, a positive allosteric modulator (PAM) of nicotinic acetylcholine receptors, as well as a cholinesterase inhibitor, improves the efficiency of coupling choline binding to channel opening; it may also preserve the receptor in a sensitive, as opposed to refractory, state. We conducted a pilot randomized, double-blind clinical trial comparing CDP-choline and galantamine to placebos in schizophrenia patients with negative symptoms who were receiving 2nd generation antipsychotics. The study was designed to evaluate the feasibility of the intervention and procedures and provide preliminary data regarding efficacy, safety and tolerability.
Methods: Patients with DSM-IV diagnoses of schizophrenia or schizoaffective disorder and had residual negative symptoms were randomly assigned to galantamine/CDP-choline or matching placebos for both for 16 weeks. Subjects were on stable doses of 2nd -generation antipsychotics for at least four weeks prior to enrollment and for the study duration. Five PANSS negative symptom items (blunted affect, emotional withdrawal, poor rapport, passive/apathetic social withdrawal, and lack of spontaneity and flow of conversation) were used to determine eligibility. A score of at least 4 (moderate) on at least one was required. PANSS positive symptom items (conceptual disorganization, hallucinations, suspiciousness, and delusions) could not exceed a total of 18. Galantamine was titrated to 24 mg/day and CDP-choline to 2000 mg/day. Symptoms were assessed at baseline, week 4, 8, 12, and 16. Treatment emergent side effects were monitored weekly. The primary measure of efficacy was the 5-item negative-symptom cluster.
Results: Forty-four participants were randomly assigned to treatment and 43 received study medication: 19 (17M; 2F) in the Galantamine/CDP-choline group and 24 (22M; 2F) in the Placebo group. Trial completion was high in both groups: Galantamine/CDP-choline 79.0%; and Placebo 79.2%. There were drop-outs due to adverse events in the Galantamine/CDP-choline group during titration, related to gastrointestinal distress, mostly nausea. There was no statistical difference in baseline characteristics between the two groups in age, education, pre-morbid IQ, racial and ethnic composition, age of first hospitalization, and negative symptom and positive symptom PANSS scores, all P values $>.05$. On average, this sample of participants tended to be older

(Galantamine/CDP-choline group: M = 54.37, SD = 8.50; Placebo group: M = 52.38, SD = 11.04) than samples commonly enrolled in clinical trials. A 2 x 5 (Group x Time) mixed effects model was used to analyze PANSS symptom outcomes. The treatment effect is determined by the Group X Time interaction. This interaction was not significant for the negative symptom cluster (P > .05) or the total positive syndrome score, (P > .05). However, there was a significant Time effect for both the negative symptom cluster (P < .0001) and positive syndrome score (P < .01), indicating a reduction in symptoms over time.

Discussion: Because of agonist-induced desensitization of the α_7 nAChR, chronic administration of directly acting agonists may be associated with functional antagonism (i.e., desensitization of receptors that may already be under-expressed). The novelty of this trial is combining a direct agonist with a PAM for the treatment of negative symptoms. Congruent with our earlier open-label case series, we found administration of the combination treatment to be both feasible and well tolerated. In the event of adverse events, experience indicates that slowing titration could reduce the likelihood of early drop-out due to gastrointestinal complaints. However, there was no efficacy signal in either our primary measure of negative symptoms or in other measures of symptom severity. Improvement over time in both groups highlights the contribution of enhanced clinical engagement inherent in a trial. The trial size makes it difficult to draw conclusions regarding efficacy of this combination. Further information from the present trial will inform the relationship, if any, between plasma concentrations of choline and expression of DNA methyltransferase 1 (DNMT-1) in white cells to outcome.

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46. Cardiovascular Effects of Folate Supplementation in Schizophrenia: An Interim Analysis

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Background: The folate hypothesis of schizophrenia suggests that the methlenetetrahydrofolate reductase (*MTHFR*) 677C/T variant, responsible for aberrant folate and homocysteine metabolism, is associated with a higher risk for atypical antipsychotics (AAPs) linked metabolic abnormalities (Ellingrod and others 2008). It is not known if folate supplementation can overcome *MTHFR's* effects and reduce AAP linked metabolic complications. The purpose of this pilot study is to examine the effects of folate supplementation on metabolic measures, endothelial functioning, and inflammatory markers in schizophrenia subjects treated with AAPs.

Methods: Subjects who were part of our phase I cross-sectional study were given 5 mg of folate daily for three months in an open fashion. Endothelial function was assessed using peripheral arterial tonometry (RHI-PAT) using the EndoPAT 2000. Baseline measurements included the RHI index, BMI, fasting metabolic laboratory measures-reactive protein, homocysteine, IL-6, and leptin and were reassessed after three months. Physical activity was assessed using the Total Activity Measure 2 (TAM2) (Orrell and others 2007).

Results: A total of 18 subjects are part of this interim analysis. Their mean age is 50.5 ± 6.03 years and racially, 61% are Caucasian and 33% are African American. Their mean BMI is 39.5 ± 10 kg/mm2, 89% met the NCEP-ATP-III criteria for metabolic syndrome and on average they met a mean of 3.05 ± 1.35 (range 2-5) criteria for metabolic syndrome. Fifty-eight percent of subjects currently smoked cigarettes and seven subjects had a *MTHFR* T allele. After 3 months of folate supplementation, the mean PAT index increased by 14% from (1.72 to 1.83, p = 0.28) indicating better endothelial functioning. Mean homocysteine levels decreased by 15% (10.7 to 9.1 umol/L, p < 0.07). Mean BMI did not change significantly between baseline and endpoint, but did decrease from 40 to 39 (p = 0.64). Mean IL-6 also decreased 30% from 4.93 to 3.43 pg/ml (p = 0.23), however mean leptin levels increased 10% from 29.7 to 32.7 ng/ml (p = 0.54). Subjects also exercised less during the study, with TAM2 scores decreasing by 15% over the 3 months (p = 0.03). At baseline 70% of subjects meet criteria for endothelial dysfunction (RHI < 1.67) and after 3 months of folate supplementation this decreased to 40% ($\chi 2 = 8.6$, p = 0.0033). Differences in the RHI index were seen in relation to smoking where non-smokers had a 45% increase in RHI, compared to a 7% increase in smokers (F = 2.8 (1,10), p = 0.12). Additionally those with a *MTHFR* CC genotype experienced an 24% increase in the RHI compared to a 1.3% decrease in those with the T allele, although this was not statistically significant (p = 0.13). This difference may indicate that folate supplementation may benefit the *MTHFR* CC genotype group the most.

Discussion: The results of this interim analysis are encouraging in terms of the endothelial and inflammatory effects of folate supplementation in schizophrenia subjects receiving AAPs. Folate use may help reduce some of the AAP linked metabolic risks and in fact we found that folate supplementation for 3 months resulted in a significant reduction in the number of subjects meeting criteria for endothelial dysfunction. Removal of this medical diagnosis may mean reductions in overall cardiovascular risk. Those with the *MTHFR* T allele (associated with reduced folate metabolism) as well as current cigarette smokers may not fully benefit from folate supplementation. Given our extremely small sample size, more work needs to be done as we continue our folate supplementation trial.

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Ellingrod VL, Miller DD, Taylor SF, Moline J, Holman T, Kerr J. 2008. Metabolic syndrome and insulin resistance in schizophrenia patients receiving antipsychotics genotyped for the methylenetetrahydrofolate reductase (MTHFR) 677C/T and 1298A/C variants. *Schizophr Res* 98(1-3):47-54.

Orrell A, Doherty P, Miles J, Lewin R. 2007. Development and validation of a very brief questionnaire measure of physical activity in adults with coronary heart disease. *Eur J Cardiovasc Prev Rehabil* 14(5):615-23.

Disclosure: V. Ellingrod: None. **T. Grove:** None. **S. Taylor:** Part 4: St. Jude Medical, Neuronetics.

47. Moderate Dose Varenicline Treatment on Neurobiological and Cognitive Biomarkers in Schizophrenia Smokers and Non-smokers

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Background: Smoking or nicotine challenge in humans transiently influences many biomarkers associated with schizophrenia, including prepulse inhibition, sensory gating, antisaccade, eye-tracking, sustained attention, information processing speed, and spatial information processing, leading to the pharmaceutical effort to target nAChRs for novel CNS drug development. There are 17 known nicotinic receptor subunits. It is unclear which nAChR subtype(s) is responsible for these seemingly pervasive

nicotinic effects: identifying it would be instrumental for guiding the development of biologically based drugs. Of the nAChR subtypes, α4β2, α3β4 and α7 are the primary ones in the brain. Until recently, clinical efforts on nAChR therapeutics for schizophrenia have been focused more on α7. No data systematically comparing clinical α4β2 nAChR action across schizophrenia-related biomarkers are available. We recruited smoking and nonsmoking schizophrenia patients to evaluate varenicline effects with and without potential smoking-related confounds. We chose a moderate dose (1 mg/day), which is half of the recommended 2 mg for smoking cessation, because the moderate dose strategy should 1) reduce risk especially in nonsmoking patients; 2) still allow testing whether sustained α4β2 modulation would influence biomarkers; and 3) further capitalize on the differential affinity of varenicline to α4β2 vs. other subunits such that significant effects, if found, are likely due to α4β2 rather than α7 or α3β4 nAChR subunits.

Methods: We investigated the effect of varenicline, a relatively specific α4β2 partial agonist/antagonist, on key biomarkers that are associated with schizophrenia and are previously shown to be responsive to nicotinic challenge in humans. The design was a double-blind, parallel, randomized, placebo controlled trial in schizophrenia patients to examine effects of vareniclineon biomarkers at short-term (2 week) and long-term (8 week), using a slow titration and moderate dosing strategy for retaining α4β2 specific effect while minimizing side effects. 69 smoking and nonsmoking patients randomized; 64 completed week 2; 59 completed week 8 of the biomarker and cogntive function tasks. The main outcome measures were prepulse inhibition, sensory gating, antisaccade, spatial working memory, eyetracking, processing speed, and sustained attention.

Results: Moderate dose of varenicline 1) reduced P50 sensory gating deficit after a long-term ($p = 0.006$) but not short-term treatment; significant in nonsmokers but not in smokers; 2) reduced startle reactivity ($p = 0.015$) regardless of baseline smoking status; and 3) improved executive function by reducing antisaccade error rate ($p = 0.034$) regardless of smoking status. Moderate dose varenicline had no significant effect on spatial working memory, predictive and maintenance pursuit, processing speed, or sustained attention by Connor's CPT. Clinically, there was no evidence of exacerbation of psychiatric symptoms, psychosis, depression, or suicidality using a gradual titration, 1 mg daily dose.

Discussion: We observed no evidence that moderate dose, 8-week varenicline is unsafe in stable, medicated schizophrenia patients. There is evidence of a long-term neurobiological improvement on sensory gating and antisaccade functions, and a nonsignificant reduction in psychotic symptoms, suggesting a unique efficacy profile of the presumed partial agonist/antagonist α4β2 nAChR modulation. These findings encourage further development of α4β2 nAChR modulating compounds and optimizing dosing and treatment duration that are safe and effective for treating specific neurobiological deficits, a critical unmet treatment need in schizophrenia.

Disclosure: E. Hong: None.

48. The Alpha7 Neuronal Nicotinic Receptor (NNR) Modulator TC-5619 had Beneficial Effects and was generally Well Tolerated in a Phase 2 Trial in Cognitive Dysfunction in Schizophrenia (CDS)

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Background: TC-5619 is a selective alpha7 NNR modulator. TC-5619 had efficacy in preclinical models of memory and schizophrenia[1] and was generally well tolerated in phase 1 trials in healthy volunteers, demonstrating a robust improvement in attention at 6.8 mg in a multiple rising dose study. This trial tested the effect of TC-5619 on cognitive and negative symptoms in subjects with schizophrenia.

Methods: In the US and India 185 outpatients (18–65 years; male 69%; 46% tobacco users) with stable schizophrenia and taking quetiapine or risperidone were randomized to 12 weeks of placebo ($n = 91$) or TC-5619 ($n = 94$: 1 mg po qd Day 1 to Week 4; 5 mg po qd Week 4 to Week 8; 25 mg po qd Week 8 to Week 12). The primary outcome measure tested executive function at Weeks 4, 8 and 12: Groton Maze Learning Task (GMLT) of the computerized CogState Schizophrenia Battery (CSB). Secondary outcome measures included: CSB composite score; Scale for Assessment of Negative Symptoms (SANS); CGI-Global Impression (CGI-I); CGI-Severity (CGI-S); Subject Global Impression–Cognition (subject-rated scale assessing Speed of Thinking, Memory & Attention). Safety measures included: AEs; physical exam; vital signs; serum and urine labs; ECG; Abnormal Involuntary Movement Scale (AIMS); Columbia Suicide Severity Rating Scale (CSSRS); and Calgary Depression Scale for Schizophrenia (CDSS).

Results: Blinded GMLT data showed a positive skew; all GMLT data were log(10) transformed. GMLT results favored TC-5619 (Hochberg adjusted $p = 0.054$) and met predefined success criteria (1-sided $p < 0.10$). SANS, CGI-I and SGI-Cog results favored TC-5619 (unadjusted $p < 0.05$) on a measurement date. The effect was driven primarily by tobacco users. TC-5619 was generally well tolerated and there were no noteworthy safety findings.

Discussion: The concordance between objective, clinician-rated and subject-rated scores supports the promise of TC-5619 for treating the cognitive dysfunction and negative symptoms in patients with schizophrenia.

[1]Hauser TA, Kucinski A, Jordan KG, Gatto GJ, Lippiello PM, Bencherif M: TC-5619: An α7 NNR selective agonist that demonstrates efficacy in animal models of schizophrenia. *Biochem Pharmacol* 1009; 78: 803-812.

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49. Daytime Sleepiness as a Mediator of Treatment Outcome in a Placebo- and Quetiapine XR-Controlled Trial of Lurasidone in Patients with Schizophrenia

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Background: Daytime sleepiness is an adverse effect observed with some antipsychotic agents. The impact of sleepiness during waking hours on treatment outcomes is under-appreciated, and infrequently assessed using a validated scale. The aim of this post-hoc analysis was to evaluate the effect of lurasidone (80 mg/d or 160 mg/d) and quetiapine XR 600 mg/d on daytime sleepiness and its mediating effects on clinical, cognitive and functional outcomes in patients with an acute exacerbation of schizophrenia.

Methods: Patients who met DSM-IV criteria for schizophrenia were randomized to 6-weeks of double-blind treatment with lurasidone 80 mg/d ($N = 125$, LUR80), lurasidone 160 mg/d ($N = 121$, LUR160), quetiapine XR 600 mg/d ($N = 120$, QXR), or placebo ($N = 122$), administered once-daily in the evening. The effect of study medication on persistent daytime sleepiness was

measured using the Epworth Sleepiness Scale (ESS). Daytime sleepiness as a mediator of reduction in cognitive or functional performance was evaluated in specific situations using the ESS items, as well as the ESS total score. Cognitive performance was assessed using CogState computerized battery composite scores. Functional performance was evaluated using the UCSD Performance-based Skills Assessment, Brief (UPSA-B). We applied a structural equation model to explore the potential mediating effect of daytime sleepiness associated with antipsychotic treatments on these outcome measures. Because cognition and UPSA-B assessments can be influenced by cultural differences, analyses were performed in all subjects and within the subgroup of US sites.
Results: Analysis of ESS change score from baseline showed that QXR (+ 0.6, SD 3.5) was associated with a statistically significant increase in daytime sleepiness, when compared to placebo (-0.9, SD 3.5) ($p = 0.001$) or lurasidone (LUR80 -1.1, SD 3.5, $p < 0.001$; LUR160 -0.7, SD 3.5, $p = 0.007$). Among the 8 common situations assessed in ESS scores, QXR was associated with a significant increase in sleepiness in 5 situations when compared to placebo ($p = 0.013$, 'dozing when talking'; $p = 0.008$, 'sitting and reading'; $p < 0.001$, 'watching TV'; $p = 0.005$, 'afternoon resting'; $p = 0.051$, 'sitting quietly after lunch without alcohol'). There was significantly greater improvement in PANSS total scores and UPSA-B performance score for all 3 active treatments compared to placebo. Significant benefit of LUR160 in overall cognitive performance was also observed when compared to placebo and QXR. Structural equation models showed that high likelihood of ESS item "dozing when talking" was a significant mediator of reduction in overall cognitive performance for QXR vs. placebo ($p = 0.043$ US sites) and for QXR vs. LUR160 ($p = 0.006$ for US sites, and $p = 0.015$ for all subjects). The overall ESS total score or the more soporific "afternoon resting" item score were not significant mediators for cognitive outcome. For the UPSA-B total score, change in ESS total score was a significant mediator for QXR vs. placebo ($p = 0.003$, US subjects only; $p > 0.05$), indicating increase in overall daytime sleepiness might mediate reduction in functional performance among QXR treated subjects, while a reverse trend was observed for those in the placebo arm. We also found overall sleepiness to be a significant mediator of changes in PANSS total score for QXR vs. placebo ($p = 0.026$, US sites only; $p > 0.05$, all subjects), but not for other treatment comparisons.
Discussion: Treatment with 80 mg or 160 mg of lurasidone, administered once-daily in the evening, was associated with a small reduction in daytime sleepiness that was similar in magnitude to placebo. In contrast, treatment with quetiapine XR 600 mg was associated with a significant increase in self-reported daytime sleepiness compared to placebo. Our findings indicate overall daytime sleepiness might be a mediator of change in clinical or performance-based functional outcomes, while sleepiness as a mediator of cognitive performance is more dependent on the specific situation for which sleepiness is reported.
Disclosure: R. Silva: Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Cucchiaro:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Pikalov:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Xu:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **C. Siu:** Part 1: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 2: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 3: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche). **A. Loebel:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Kalali:** Parts 1-5: Full-time employee of Quintiles Inc.

50. Clinical Trials of Potential Cognitive-Enhancing Drugs in Schizophrenia: What Have We Learned So Far?

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Background: A large number of studies have been conducted or are currently underway to examine the treatment of cognitive impairment associated with schizophrenia (CIAS). In light of the number of negative studies, we critically reviewed completed and ongoing CIAS trials to examine factors that may contribute to this lack of success.
Methods: Trials were identified through searches of the website 'www.clinicaltrials.gov' through 20 April 2011. We included trials that were conducted in people with schizophrenia, the effects on cognition were either a primary or secondary outcome, and the effect of a pharmacologically active substance was examined. Drug challenge, pharmacokinetic, pharmacodynamic or prodrome of psychosis studies were excluded. We also evaluated whether the study had sufficient statistical power (beta = 0.80) to identify true treatment differences based upon the following assumptions: two-tailed alpha of 0.05, test-retest of the primary outcome of Intra-Class Correlation (ICC) = 0.90 [consistent with the MATRICS Consensus Cognitive Battery (MCCB) composite score in multi-site studies] and effect size estimates of $d = .5$ (medium effect).
Results: We identified 118 trials; of these, 61 were completed and 57 are ongoing. Seventy-three (62%) used an add-on or "co-treatment" parallel group design. Fifty per cent of completed add-on trials and 22.0% of trials using other designs had adequate study design details available in the public domain and were included in our analysis. A similar distribution of sample sizes was observed for both completed trials and ongoing trials. However, only 17.6% of completed and 35.9% of ongoing trials report a sample size that was or is anticipated to be sufficient to produce statistical power to detect a medium ($d = 0.5$) effect size, which requires 71 subjects per group (given a 2-arm trial with drug and placebo). The trial duration was >8 weeks in 41.2% of completed trials, but there appears to be a pattern of longer duration among ongoing trials, with 66.7% being >8 weeks long. No clear preference or consistency in the primary neurocognitive outcome measure was observed among completed add-on trials, with 14.7% using the MCCB. Twenty one (53.8%) ongoing add-on trials use the MCCB, either alone or in combination with another neurocognitive assessment battery. No clear pattern could be established in the choice of co-primary outcome related to functioning or functional capacity in either completed or ongoing trials. The large majority of completed and ongoing trials were performed in participants who tended to be ≥65% male, with chronic, stable schizophrenia. Five (8.8%) ongoing trials are being specifically conducted in subjects with recent onset schizophrenia. A defined level of cognitive impairment was used as an inclusion criterion in 20.6% of the completed and 15.4% of the ongoing add-on trials. However, there is no consistency in the definitions of cognitive impairment used. Based on the available data, the subjects in the completed trials had at least a minimal level of cognitive impairment. Among the 17 completed trials with available outcome information, 16(94%) reported lack of separation between a drug and placebo on a cognitive endpoint.
Discussion: Since half of the completed trials do not have results in the public domain, and even fewer in the peer-reviewed literature, it is unfortunately challenging to make any reliable appraisals of the factors that may be associated with failure of drugs to separate from placebo. The possible methodological reasons are varied. Cross-over designs for cognitive outcomes are appealing due to their potential capacity to enhance power through within-subjects analyses, but these trials may obscure effects that could be detected in parallel group designs because practice and treatment effects

may be confounded. Studies completed to date have not had sufficient statistical power to state confidently that a particular treatment does not have potential efficacy. Further, the predominant patient population in these studies has been older, chronic, and mostly male, who may be the least likely to benefit from cognitive enhancement. A substantial number of clinical trials of potential treatments for cognitive enhancement in schizophrenia are currently ongoing. These studies, with larger and more diverse samples, also including several trials specifically focusing on recent onset schizophrenia, are likely to shed more light on the challenges of CIAS trial design and methodology, and may increase the probability of identifying treatments with beneficial effect on cognitive impairment in schizophrenia.

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51. Adjunctive Lisdexamfetamine Dimesylate Treatment of Predominant Negative Symptoms of Schizophrenia in Clinically Stable Adults Maintained on Atypical Antipsychotic Agents: a 14-Week Trial

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Background: Treatments are needed for negative symptoms of schizophrenia (NSS), associated with low mesocortical tract dopamine activity. This multicenter study examined adjunctive treatment with lisdexamfetamine dimesylate (LDX; Vyvanse®, Shire US Inc.), a d-amphetamine prodrug, to antipsychotics in clinically stable adults with predominant NSS.

Methods: Outpatients with stable schizophrenia ($\geq$2 years), predominant NSS (SANS-18 [items 1-6, 8-12, 14-16, 18-21] score $\geq$55, score $\geq$3 on $\geq$2 SANS Global items, PANSS positive score $<$20), and maintained on stable atypical antipsychotic ($\geq$12 weeks) underwent 3 weeks screening, 10 weeks open-label (OL) LDX augmentation (20-70 mg/d, and then 4 weeks double-blind randomized withdrawal (RW). Eligible participants (any SANS-18 improvement at week 10) entered double-blind, placebo-controlled RW. Efficacy measures included SANS-18 (primary) and SANS Global items, PANSS total and subscales. Safety evaluations included treatment-emergent adverse events (TEAEs), vital signs, and Calgary Depression Scale for Schizophrenia (CDSS). Withdrawal criteria included $\geq$25% increase in PANSS total or $\geq$2-point increase on positive items at 2 consecutive visits, increase in suicidal ideations, or positive drug screen.

Results: 92 participants received OL LDX; 69 entered RW to continue LDX (n = 34) or placebo (n = 35); 13 were discontinued during RW.

At baseline (week 0), mean (SD) SANS-18 score was 60.2 (4.36). Mean change (95% confidence interval [CI]; weeks 0-10 OL) was -12.9 (-15.0, -10.8)(primary endpoint; $P<.0001$). 52.9% were SANS-18 responders ($\geq$20% reduction from baseline). All SANS Global items showed significant decreases ($P<.0001$ for each). PANSS total and positive score mean changes (95% CI) were -9.8 (-11.7, -8.0) and -1.0 (-1.4, -0.5). During RW (weeks 10-14), no differences (change from randomization baseline) were found between LDX and placebo in SANS-18 or PANSS positive scale scores. Change (weeks 0-10) in systolic and diastolic BP were 2.6 (8.13) and 2.3 (7.12) mmHg and pulse was 5.1 (11.74) bpm. There was no meaningful change in CDSS (week 0-14) for LDX and placebo. In OL, TEAE incidence was 60.9% and TEAEs $\geq$5% were headache (14.1%), decreased appetite (10.9%), insomnia (10.9%), dizziness (8.7%), dry mouth (6.5%), and diarrhea (5.4%). Serious TEAEs occurred in 3.3%; 5.4% discontinued due to TEAEs in OL.

Discussion: NSS significantly decreased and PANSS total score improved with OL LDX without positive symptom worsening. There was no overall symptom worsening with abrupt LDX discontinuation during placebo treatment. LDX may be safely administered to carefully selected patients with clinically stable schizophrenia. Confirmation with larger, controlled trials is needed.

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Disclosure: R. Lasser: Part 1: Shire employee and holds stocks and/or stock options in Shire, Part 2: Shire employee and holds stocks and/or stock options in Shire, Part 3: Shire employee and holds stocks and/or stock options in Shire, Part 5: Shire employee and holds stocks and/or stock options in Shire. **B. Dirks:** Part 1: Shire employee and holds stocks and/or stock options in Shire and Johnson & Johnson, Part 2: Shire employee and holds stocks and/or stock options in Shire and Johnson & Johnson, Part 3: Shire employee and holds stocks and/or stock options in Shire and Johnson & Johnson, Part 5: Shire employee. **H. Nasrallah:** Part 1: Over the past two years, I have conducted clinical research with the following companies, with all funding being sent directly to my institution [University of Cincinnati]: Forest, Janssen, Otsuka, Pfizer, Roche, Shire, Part 2: Over the past 2 years, I have personally received consulting fees or speaking honoraria exceeding $10,000 from the following companies: Astrazeneca, Janssen, merck, Novartis, Pfizer, Sunovion. These payments represented more than 5% of my total annual income, Part 3: None of the research grants were sent to me personally. As a full-time university employee, all grants are property of the university. As shown in part two above, I only received honoraria and consulting fees from the companies listed above., Part 4: As above in Part three. Also, neither I or my family members own any stocks in any pharmaceutical company listed or mot listed in this disclosure, Part 5: Not applicable. I am a full-time university employee. **C. Kirsch:** Part 1: Shire employee and holds stocks and/or stock options in Shire, Part 2: Shire employee and holds stocks and/or stock options in Shire, Part 3: Shire employee and holds stocks and/or stock options in Shire, Part 5: Shire Development Inc. **J. Gao:** Part 1: I am an employee of Shire Development Inc. and hold stocks and stock options of the company (Shire plc) as a result., Part 2: Salary, bonus, and exercise of stocks/options at Shire., Part 3: Salary, bonus, and exercise of stocks/options at Shire., Part 5: Shire Development Inc. **M. Knesevich:** Part 1: Over

the past two years I have conducted clinical research for and received research grants from the following pharmaceutical companies: Astra Zeneca Pharma, Bioline Rx, Cephalon Pharmaceuticals, Danone Corporation, Forest Research Institute, Janssen Pharma, Johnson & Johnson Pharmaceuticals, Merck, Novartis Inc., Pfizer Pharmaceuticals, Roche Pharma, Shire Pharmaceuticals, Part 2: Over the past two years I have received research grants greater than $10,000 per year from the following companies: Astra Zeneca Pharma, Bioline Rx, Cephalon Pharmaceuticals, Danone Corporation, Forest Research Institute, Janssen Pharma, Johnson & Johnson Pharmaceuticals, Merck, Novartis Inc., Pfizer Pharmaceuticals, Roche Pharma, Shire Pharmaceuticals, Part 3: I have served as a contract clinical investigator with the following pharmaceutical companies and the clinical research grants paid to me represent more than 5% of my personal income calendar years 2009 to present: Astra Zeneca Pharma, Bioline Rx, Cephalon Pharmaceuticals, Danone Corporation, Forest Research Institute, Janssen Pharma, Johnson & Johnson Pharmaceuticals, Merck, Novartis Inc., Pfizer Pharmaceuticals, Roche Pharma, Shire Pharmaceuticals, Part 4: Astra Zeneca Pharma, Bioline Rx, Cephalon Pharmaceuticals, Danone Corporation, Forest Research Institute, Janssen Pharma, Johnson & Johnson Pharmaceuticals, Merck, Novartis Inc., Pfizer Pharmaceuticals, Roche Pharma, Shire Pharmaceuticals, Part 5: My primary employer is the company I own, University Hills Clinical Research. **J. Lindenmayer:** Part 1: Advisory Board/Consultancy (fees paid to Institution): Roche; Merck; Janssen; Lilly; Shire; Multi Health Systems (Rating Scale Royalties), Part 2: Office of Mental Health, NY State (full time employee), Part 4: Lilly; Roche; Otsuka; Sunovion; Astra-Zeneca; Janssen.

52. Relapse Prevention with Lurasidone vs. Quetiapine XR in Chronic Schizophrenia: Results of a 12-Month, Double-Blind Study

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Background: This was a multiregional study designed to evaluate the efficacy and safety of flexible once-daily doses of lurasidone (40-160 mg) vs. quetiapine XR (QXR; 200-800 mg) in preventing relapse in subjects with chronic schizophrenia who demonstrated a clinical response after completing an initial double-bind, 6 week trial of lurasidone (80 mg; 160 mg), QXR (600 mg), and placebo.
Methods: Subjects who completed the initial 6-week trial enrolled in a 12-month double-blind extension phase study in which subjects initially treated with lurasidone continued on flexible once-daily doses of lurasidone (40-160 mg; $n = 151$), and subjects initially treated with QXRcontinued on flexible once-daily doses of QXR (200-800 mg; $n = 85$). Subjects initially treated with placebo were started on flexible once-daily doses of lurasidone (40-160 mg; $n = 56$), and were analyzed separately. Relapse was defined as the earliest occurrence of (1) $\geq 30\%$ increase in the PANSS total score from the end of acute phase score and a CGI-Severity score ≥ 3; (2) hospitalization for worsening psychosis; or (3) emergence of suicidal ideation, homicidal ideation and/or risk of harm to self or others. The primary endpoint, time-to-relapse, was analyzed using a Cox proportional hazards model with country as a covariate. The pre-specified non-inferiority margin for the risk of relapse hazard ratio was 1.93. Safety and tolerability measures included adverse events (AEs), body weight, lipid parameters, and ECGs.
Results: The Kaplan-Meier estimate of the probability of relapse at 12 months was lower on lurasidone vs. QXR (0.237 vs. 0.336). The risk of relapse hazard ratio [95%-CI] based on the Cox model was 0.728 [0.410, 1.295], indicating a 27.2% reduction in risk of relapse compared to QXR. Treatment with lurasidone was associated with a significantly greater change from acute phase baseline in PANSS total scores at Months 3 (-32.1 vs. -27.4; $p = 0.022$), 6 (-34.1 vs. -26.8; $P \leq 0.001$), 9 (-33.4 vs. -24.8; $p = 0.003$), and 12 (-34.6 vs. -25.7; $p = 0.006$), based on an MMRM analysis. Treatment with lurasidone was also associated with a greater change from acute phase baseline in the MADRS total scores at Months 6 (-6.2 vs. -4.8; $p = 0.07$) and 12 (-6.0 vs. -3.8; $p = 0.04$). The mean daily dose of lurasidone was 125.5 mg and the mean daily dose of QXR was 629 mg. Discontinuation due to insufficient clinical response was lower in the lurasidone group compared to the QXR group (9% vs. 21%). Discontinuation rates due to AEs were similar for lurasidone and QXR (7% vs. 5%). AEs on lurasidone with an incidence $\geq 5\%$ were akathisia (12.1%), headache (9.2%), parkinsonism (8.7%), insomnia (7.7%), nausea (6.3%), and anxiety (5.3%). For lurasidone and QXR, respectively, mean change (from acute phase baseline, OC analysis) in weight (kg)at 6 months was +0.8 vs. +6.5, and at 12 months was -0.08 vs. +1.0. Median change in glucose (mg/dL) at 6 months was -2.0 vs. +2.0, and at 12 months was -0.5 vs. +1.0. Median change in cholesterol (mg/dL) at 6 months was -8.0 vs. +6.0, and at 12 months was 0.0 vs. +4.0. Similarly, median change in triglycerides (mg/dL) at 6 months was -4.0 vs. +6.0, and at 12 months was -18.0 vs. -7.0. There were no clinically meaningful changes in vital signs, or laboratory and ECG parameters on either drug.
Discussion: This double-blind study demonstrated the non-inferiority of lurasidone to QXR in risk for relapse over a 12 month period. Treatment with lurasidone was associated with a 27.2% reduction in risk of relapse compared to QXR (hazard ratio, 0.728). Lurasidone treatment was associated with significantly greater improvement on the PANSS total score and MADRS from acute phase baseline to 12 month study endpoint. Treatment with lurasidone was also associated with few adverse effects on metabolic parameters, and a minimal effect on weight and prolactin.
Disclosure: A. Loebel: Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Cucchiaro:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Xu:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **K. Sarma:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Pikalov:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Kalali:** Parts 1-5: Full-time employee of Quintiles, Inc.

53. Paternal Age and Treatment Response in Adolescents with Schizophrenia

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Background: Advanced paternal age (APA) is associated with increased risk for schizophrenia, particularly sporadic cases. Twenty-five percent of schizophrenia cases may be explained by advancing paternal age. Several reports show distinct illness features in cases with APA, suggesting APA may contribute to a specific subtype of schizophrenia.[1,2,3] Treatment response with respect to APA has not been examined.
Methods: We conducted a post-hoc analysis of data from a pharmacological trial in adolescent schizophrenia cases (NCT 00518323). The main efficacy and safety results from this study are reported elsewhere.[4] The randomized 6-week double-blind trial assessed treatment response to placebo and 3 different weight-based doses of paliperidone extended-release (ER). We examined the correlation of parental ages with (1) age of onset, (2) symptom severity, and (3) response defined as a 20% or more improvement in PANSS total score from baseline ratings. Parental age was defined as age (in years) at the birth of the child. The analytic approach employed correlations, analysis of covariance, linear regression and logistic regression.

Results: Paliperidone ER was effective in treating schizophrenia at doses of 3-12 mg/day in 201 adolescents (age 12-17), as previously reported.[4] Mean (SD) for paternal age was 29.2 (6.2), range (16-50); maternal age was 26.8 (5.7), range (17-42). The new analyses showed: (1) Parental age did not significantly correlate with age of onset or with initial symptom severity. (2) Both paternal and maternal ages were significantly correlated with response; offspring of older parents in both active and placebo treatment groups exhibited a higher rate of response. Significant predictors of response were: study medication ($p = .008$), paternal age ($p = .015$) and maternal age ($p = .015$). Accounting for treatment groups (placebo vs. all paliperidone ER arms), both maternal and paternal age showed significant effect ($p < .03$) on response. Separating treatment groups into placebo vs. 1.5 mg paliperidone ER (ineffective dose) vs. the combined effective doses 3-12 mg, paternal age showed a robust effect ($p = .031$) on response, with only a trend association to maternal age ($p = .065$).
Discussion: These results show increasing parental age is associated with greater response in adolescents. The findings also augment the hypothesis that advanced paternal age contributes to a specific subtype of schizophrenia, consistent with earlier reports by Malaspina *et al.*[1,2] This finding offers new promise for patient specific research and individualized treatment interventions. Notably, although the risk to offspring for any individual father may be increased as he ages, most schizophrenia cases with advanced paternal age-related illness are born to fathers in their 30's. The risk for schizophrenia is only doubled for fathers of this age, but far more men have children at these ages than at older years.
Disclosure: D. Malaspina: None. **O. Mark:** None. **S. Gopal:** Part 1: Full time employee Johnson & Johnson, Part 2: Shareholder Johnson & Johnson, Part 3: Full time employee Johnson & Johnson, Part 4: Not applicable, Part 5: Full time employee Johnson & Johnson. **I. Nuamah:** Part 1: I am a full-time employee of Johnson & Johnson Pharmaceutical Research and Development, Part 2: I am a full-time employee of Johnson & Johnson Pharmaceutical Research and Development, Part 5: Johnson & Johnson Pharmaceutical Research and Development. **A. Savitz:** Part 4: When employed by Weill Cornell Medical College (left May 2011) had research support from: Sanofi-Aventis Abbott Laboratories, Part 5: Johnson & Johnson Pharmaceutical Research and Development. **J. Singh:** Parts 1-5: Full time employee Johnson & Johnson PRD LLC. **D. Hough:** Parts 1-5: Employee of Johnson & Johnson Pharmaceutical Research and Development LLC.

54. Correlation between Antipsychotic Efficacy and Weight Gain with Iloperidone in Short and Long-term Trials in Schizophrenia

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Background: Antipsychotic agents are associated with varying degrees of weight gain. Several published studies have observed that improvement of psychosis in schizophrenia is associated with increased Body Mass Index (BMI). We examined whether a correlation exists between weight gain and efficacy (decreased psychosis score) in short- and long-term clinical trials of iloperidone, one of the new atypical antipsychotics approved for the treatment of schizophrenia in adults.
Methods: Two sets of clinical trial data were analyzed: 1) 4 randomized, double-blind, placebo-controlled short-term trials of 4- or 6-weeks duration comparing iloperidone 4-24 mg/d to placebo in patients with schizophrenia and 2) three randomized, long-term phase 3 trials lasting up to 52 weeks that compared iloperidone 4-16 mg/d with haloperidol 5-20 mg/d in patients with schizophrenia. The intent-to-treat population, containing all randomized patients who received at least one dose of double-blind study medication and had at least one efficacy measurement, was used for analysis. For each visit, change from baseline in Positive and Negative Syndrome Scale total (PANSS-T) score (calculated as baseline value minus post-baseline value, decreased score indicates clinical improvement) were obtained. Correlation coefficients and corresponding *P* values were calculated from Pearson correlation analysis of change from baseline in PANSS-T and change from baseline in weight (kg).
Results: 1) In the short-term 4 and 6 week studies, no statistically significant correlations between weight gain and efficacy were observed for any iloperidone treatment group at study endpoints. Pearson correlation coefficients for Week 4 were -0.0420 for iloperidone 4-8 mg/d ($P = 0.59$), 0.0094 for iloperidone 10-16 mg/d ($P = 0.88$), and 0.0548 for iloperidone 20-24 mg/d ($P = 0.37$). Corresponding correlation coefficients at Week 6 for these groups were 0.0479 ($P = 0.61$), -0.1176 ($P = 0.11$), and 0.0550 ($P = 0.66$).
2) For the long-term 52 week data, both antipsychotic agents showed similar efficacy as measured by the PANSS-T; least-squared mean (LSM) changes from baseline to Week 52 were 32.3 for iloperidone and 31.2 for haloperidol. Pearson correlation coefficients at Week 52 of 0.1687 for iloperidone ($P = 0.0002$) and 0.1972 for haloperidol ($P = 0.0099$) indicated a statistically significant relationship between weight increase and reduction (improvement) in PANSS-T score.
Discussion: A statistically significant but weak positive correlation was seen between PANSS-T efficacy and weight gain with iloperidone and haloperidol in the long-term studies only, with no correlation observed in the short-term iloperidone studies. The implications of these findings will be discussed.
Disclosure: H. Nasrallah: Part 1: Over the past two years, I have conducted clinical research with the following companies with all funding being sent directly to my institution (University of Cincinnati: Forest, Janssen, Otsuka, Pfizer, Roche, Shire, Part 2: Over the past two years, I have personally received consulting fees or speaking honoraria exceeding $10,000 from the following companies: AstraZeneca, Janssen, Merck, Novartis, Pfizer, Sunovion. These payments represented more than 5% of my total annual income, Part 3: None of the research grants were sent to me personally. As a full-time university employee, all grants are property of the university. As shown in part two above, I only received honoraria and consulting fees from the companies listed above, Part 4: As above in Part Three. Also, neither I or my family members own any stocks in any pharmaceutical company listed or not listed in this disclosure, Part 5: Not applicable. I am a full-time university employee. **M. Hochfeld:** Part 5: Novartis Pharmaceuticals. **X. Meng:** Part 5: Novartis Pharmaceuticals. **R. Wu:** Part 5: Novartis Pharmaceuticals. **A. Winseck:** Part 5: Novartis Pharmaceuticals. **S. Ahmed:** Part 5: Novartis Pharmaceuticals.

55. Examining Methods for Computing "Clinical Response" in Placebo Controlled Trials of Antipsychotics in the NEWMEDS Repository

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Background: Recent draft EMA guidelines for drug development in schizophrenia have recommended the presentation of responder analysis and have established 30% change from baseline on the PANSS as the criteria for clinical response. A 20% change has been the commonly accepted threshold for response and has been shown to correspond with a 1 point change on the CGI-S and a CGI-I of "minimally improved" and 50% PANSS reduction with "much improvement". The clinical meaningfulness of these cut-off

values may vary since 20% is clinically important for treatment-refractory patients but may not be for non-refractory patients. We examined extent of clinical response using 20% and 30% thresholds in 29 placebo controlled trials of second generation antipsychotics.

Methods: The NEWMEDS repository includes patient anonymized data from AstraZeneca, Janssen, Eli Lilly, Lundbeck, and Pfizer from 29 placebo-controlled trials of second-generation antipsychotics (placebo, n = 2200; active treatment, n = 6971) in schizophrenia, all of which demonstrated active treatment to be superior to placebo. Nineteen studies were 6 weeks long, four were 8 weeks long, three 4 weeks long, one 7 weeks long, one 12 weeks long and one was a one year study. For the purposes of analysis, all studies were truncated at 6 weeks, and one study, where this was not possible, was excluded from the analysis. We examined study results using clinical response criteria of 20% and 30% change from baseline and applied the two methods used in computing PANSS change. The unadjusted method ((change/baseline)*100) and the more psychometrically correct adjusted method ((change/(baseline-30))*100) that accounts for the fact that the PANSS lowest score is 30 (30 items, minimum per item 1). Analyses were done using last observation carried forward (LOCF). Percent of responders on placebo and active treatment based on different methods were examined as were the proportion of active treatment and placebo comparisons showing a significant difference.

Results: Comparing placebo (n = 2024) to active treatment (n = 6510), *unadjusted* response at the 20% level was attained by 30% vs. 46%, and at the 30% level by 19% vs. 29%. *Adjusted* response at the 20% level was attained by 40% vs. 57% and at the 30% level by 31% vs. 46%. Within studies, in 48% (39 of 81) of active arm comparisons to placebo, active arm had significantly higher proportion of responders at all but the 30% unadjusted level. At the 30% unadjusted level it was reduced to 30% (24 of 81). In 1 active treatment arm at 30% unadjusted, response to placebo was significantly better than active treatment. In the remaining comparisons there were no significant differences between any active treatment arms and placebo (unadjusted 20% level, n = 42/81, 30% level, n = 56/81; and adjusted 20% level, n = 44/81, 30% level n = 44/81). When examining studies, in 39% (11 of 28) no active treatment arms were significantly better than placebo on response at 20% adjusted and unadjusted and 30% adjusted. At the 30% unadjusted response level, 46% (13 of 28) of studies had no active treatment arms that were significantly better than placebo.

Discussion: In all studies, the planned analysis using PANSS as a continuous measure found active treatment to be significantly superior to placebo on at least some of the active treatment arms. Though not powered for responder analysis, number of responders was analyzed as a secondary parameter. EMA guidelines were not specific as to how change should be computed. In this large dataset of placebo-controlled studies, analyses using unadjusted 30% change showed substantially less significant differentiation between active vs. placebo (30%) as compared to adjusted 30% and adjusted and unadjusted 20% change (all three 46%-48%). Studies should be explicit as to whether they used the psychometrically preferred adjusted change or unadjusted change as this can alter results and will allow comparisons between studies.

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56. Time Course of Dropout Rates in Schizophrenia Trials Conducted from 1966 to 2010: A Systematic Review and Meta-analysis

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Background: High dropout rates have been reported in clinical trials of antipsychotic drugs, as well as in psychopharmacotherapy (Kemmler *et al.*, 2005; Cramer and Rosenheck 1998). Despite this widely observed trend, evidence-based evaluations of its time course and potential moderators are still quite limited. The objective of this study is to conduct a systematic review and meta-analysis of dropout rates in double-blind, randomized controlled trials in schizophrenia carried out between 1966 and 2010.

Methods: We searched the MEDLINE database for RCTs (randomized controlled trials) published between 1966 and 2010, supplemented by other electronic databases and hand searches. We identified 1643 unique publications from which 49 eligible studies on patients with schizophrenia or schizoaffective disorder were included in the analysis. Meta-analysis was based on data extracted from published reports of these 49 eligible RCTs, which included patient characteristics, trial design and clinical variables. In fixed dose studies, we selected the treatment arm that had shown positive findings, defined as significantly different from placebo. Risk ratio (RR), risk difference (RD), and NNTp (number needed to treat to prevent 1 outcome) were used as effect measures comparing placebo (PBO) and active antipsychotic drugs (DRUG) as a group (N = 12 atypicals, 17 conventional agents), for all-cause dropout rates in short-term (2–12 weeks) trials. The proportions of subjects who dropped out within the PBO and DRUG arms were also analyzed. Effect measures were pooled across studies using the Der-Simonian and Laird random-effects model. The quality of RCTs analyzed was evaluated using a validated omnibus rating of overall quality.

Results: Our findings indicate significant heterogeneities in dropout rates within and between PBO and active antipsychotic DRUG arms across trials ($p < 0.01$, Q-statistics for test of heterogeneity). The dropout rate in the PBO arm was significantly higher than that in the DRUG arm (RR = 1.36, 95%CI 1.27 to 1.47), with NNTp of 7.5 (RD = 13.3%) (95% CI for NNTp 6.0 to 10.0). Within the PBO arm, the overall dropout rate was 44.6% (95% CI 37.6% to 51.5%). The attrition rate in the PBO arm increased significantly over time, from 16% before 1980 to 59% between 1990 to 1995 ($p < 0.001$), followed by a significant decrease (48% between 2006 and 2010) ($p < 0.001$). The overall attrition rate for the DRUG arm was 6% before 1980, increased to 36% between 1990 and 1995 ($p < 0.001$), and then leveled off to 37% between 2006 and 2010. Study year was a significant moderator for placebo-drug difference in dropout rates, with the estimated risk difference between the PBO and DRUG arms increased between 1970 and 1990 ($p = 0.02$), and then followed by a significant decrease ($p = 0.03$). We also found study quality to be a significant moderator of RD in dropout rates, with RCTs of higher overall quality associated with increased RD favoring the active antipsychotic treatment arms.

Discussion: High dropouts have posed a major challenge to the design, analysis and interpretation of RCTS in psychiatric disorders such as schizophrenia. Our findings suggest there has been a decrease in drug-placebo difference in dropout rates since 1995, possibly due to decreased dropout rates in the placebo arm. We found both study year and study quality as significant moderators of dropout rate differences between placebo and antipsychotic treatments in schizophrenia. Further studies to verify these and other potential moderators of dropout rates are warranted, to mitigate the considerable challenge these pose to psychotherapeutic development.

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Cramer JA, Rosenheck R. Compliance with medication regimens for mental and physical disorders. *Psychiatr Serv* 1998;49:196-201.

Disclosure: O. Agid: Part 1: Advisory Board: Janssen-Ortho (Johnson & Johnson); Eli Lilly Inc.US; Eli Lilly Canada; Sepracor Speaking engagements: Janssen-Ortho (Johnson & Johnson); Eli Lilly Inc. US; Eli Lilly Canada; Novartis; Sepracor Inc., US; Sunovion, US, Part 4: Pfizer, Inc.; Janssen-Ortho (Johnson & Johnson). **C. Siu:** Part 1: Paid consultant for Pfizer, Inc.; Sunovion US (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 2: Paid consultant for Pfizer, Inc.; Sunovion US (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 3: Paid consultant for Pfizer, Inc.; Sunovion US (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche). **R. Zipursky:** Part 1: Advisory Boards: Sepracor Pharmaceuticals Canada (Sunovion Pharmaceuticals Canada); Hoffmann-La Roche Speaking engagements: Hoffmann-La Roche, Part 4: Hoffmann-La Roche. **G. Remington:** Part 1: Consultant fees - Roche Speaker Fees - Novartis Research Support - Medicure, Neurocrine Biosciences; Novartis.

57. Should Patients with Long Durations of Untreated Psychosis be included In Studies of First Episode Schizophrenia?

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Background: Current practice is to perform treatment studies of schizophrenia separately with first episode and multi-episode patients due to differences in treatment responsivity. However, the point at which treatment response changes along the illness continuum is unknown. First episode studies vary in their inclusion criteria regarding patients who have had a very long period of psychotic illness before starting treatment. Social vocational outcomes are of increasing interest in first episode studies (e.g. the NIMH RAISE initiative). This analysis examined whether first episode patients with long duration of untreated psychosis (DUP) compared with subjects with shorter DUP have different social vocational outcomes in response to treatment.

Methods: We performed a secondary analysis of data from a study of 112 subjects aged 16 to 40 years old with a first episode of schizophrenia, schizophreniform or schizoaffective disorder who were randomly assigned to treatment with olanzapine (2.5 to 20 mg daily) or risperidone (1 to 6 mg daily). Subjects were required to have less than 16 weeks of cumulative lifetime exposure to antipsychotic treatment (most had much less; 78% were antipsychotic medication naïve before study entry) but could have any duration of DUP. Follow-up lasted a total of 3 years, regardless of whether a subject stayed on randomized medication or required alternative antipsychotic treatment due to lack of response or other reasons. Functioning was assessed with the Multidimensional Scale of Independent Functioning (MSIF). Ratings for each domain varied from 1 (normal functioning) to 7 (total disability). Repeated measures analysis of covariance, using a mixed model approach, was used to examine the longitudinal patterns of functioning. The five models examined the global ratings for work, education, a composite work and education role, residential and global functioning. In a previous analysis, we included the following factors in each of the models: the two medications, time, two study sites, sex, age, alcohol use before randomization and marijuana use before randomization. For this secondary analysis, each subject was classified as having a DUP of 4 years or less or more than 4 years. A DUP group by time interaction was added to our previous models. We examined outcomes during all study periods and also for the period when subjects were prescribed their randomized antipsychotic.

Results: Two subjects dropped out before their DUP could be firmly established. The 110 subjects with DUP data were young (mean age 23), mostly male (70%), of diverse ethnic backgrounds and usually from lower class to low middle class socioeconomic backgrounds. Mean DUP was 113 (95% CI 83 to 143) weeks; the median was 48 weeks. Twenty four subjects (22% of the sample) had a DUP of longer than 4 years. The mean weeks before starting another antipsychotic was 77.1 weeks (95% CI: 60.4, 93.8) for subjects randomized to olanzapine and 87.4 weeks (95% CI: 70.4, 104.6) for subjects randomized to risperidone. The analyses revealed that the DUP group by time interaction was not significant in any of the models for either all study periods or during the period when subjects were prescribed their randomized antipsychotic. Further, adding the DUP group by time interaction did not change the overall findings from our original analyses about the effects of the other variables of interest obtained from the models without the DUP by time factor. Subsequent analyses included DUP group but not a DUP group by time interaction in the models. During the period of assigned treatment, subjects with longer DUP compared with other subjects had overall worse global MSIF ratings ($F = 4.28$, $df = 1,102$, $p < 0.04$; least square means estimated global scores for subjects with long DUP were 4.34 and 4.00 for other subjects). MSIF scores did not differ between DUP groups for the other models during assigned treatment. For all study periods, subjects with longer DUP compared with other subjects had poorer global ($F = 5.52$, $df = 1,102$, $p < 0.02$) educational ($F = 4.97$, $df = 1, 96$, $p < 0.03$) and residential ($F = 3.93$, $df = 1,102$, $p < 0.05$) functioning.

Discussion: Our results suggest that first episode subjects with long DUP compared with other subjects have poorer social vocational functioning in some domains. However, they improve with treatment to a similar degree as other subjects. Subjects with long DUP should therefore not be excluded from first episode studies focusing upon social vocational outcomes.

Disclosure: D. Robinson: Part 4: Bristol-Myers Squibb, Janssen. **S. Sunday:** None.

58. PROACTIVE: Initial Results of an RCT Comparing Long-Acting Injectable Risperidone to 2nd Generation Oral Antipsychotics

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Background: Treatment of schizophrenia requires long term interventions to reduce relapse risk. Long acting injectable (LAI) antipsychotics are a potentially valuable strategy to achieve this goal because they simplify medication administration regimens

and a missed injection is an early warning of nonadherence well before symptom exacerbation. However, their use is generally limited to patients who have clear histories of nonadherence and repeated relapse. The availability of the first 2nd generation LAI, risperidone (RISP) microspheres, led us to study it in comparison to 2nd generation oral antipsychotics in long-term treatment in a broad population of patients with schizophrenia. The PROACTIVE study tested the hypothesis that time to relapse will be longer in patients randomly assigned to receive LAI RISP than in those who receive oral 2nd generation antipsychotics.

Methods: Patients with schizophrenia or schizo-affective disorder at eight academic sites in the United States were randomly assigned to either LAI RISP or any oral 2nd generation oral antipsychotic. Primary inclusion criteria included; age 18 – 65, a symptom exacerbation within the past 12 months, living in the community for at least 4 weeks and a Clinical Global Impressions (CGI) score of at least 4 (moderately ill). Study duration for subjects ranged between 17 and 30 months depending on when they entered the study. Treatment was open after randomization. Injections and oral medication were provided at biweekly visits that included brief monitoring of symptoms and vital signs. Blinded, centralized Master Raters interviewed subjects quarterly via live two- way video over the internet to assess psychopathology with the Brief Psychiatric Rating Scale (BPRS) and the Scale for Assessment of Negative Symptoms (SANS). An independent and treatment blinded committee determined relapse defined by rehospitalization and sustained increases in psychopathology.

Results: 305 subjects were randomly assigned to treatment (153 LAI RISP and 152 oral) Mean age was 38.2; 71% were male. Racial distribution was 68.2% Caucasian and 29.8% African American. Nineteen percent were Hispanic. Education level was: at least some college 22%, high school graduate 27% and less than high school graduate 28%. 63% had never been married and 81% were not employed at study entry. Case conference consensus diagnosis was 68% Schizophrenia, 32% Schizoaffective Disorder. Mean age at first hospitalization was 22.3, mean number of prior hospitalizations was 11 and the time since the last hospitalization was 32 months. At study entry, 275 were receiving a 2nd generation oral antipsychotic, 21 LAI RISP, 32 a 1st generation oral, 10 a 1st generation injectable and 36 (12%0 were taking no antipsychotic medication. Study medication exposure. LAI RISP modal dose mg q 2 wks 37% 50, 24% 37.5, 22% 25. 5% 12.5., 12% other. Modal oral medication: risperidone 45%, olanzapine 19.2%, aripiprazole 14.6%, ziprasidone 9.3% paliperidone 6%, quetiapine 5.3%. Outcomes. There were no significant differences in time to 1st relapse between treatment groups (Log-Rank Chi Square 2.22, df 1, p 0.14) or 1st psychiatric hospitalization (Log-Rank Chi Square .982, df 1, p 0.33). However, there were significant differences favoring LAI RISP in BPRS Psychosis cluster ratings by both the non-blinded on site raters (Treatment F 4.49 df 1,289 p 0.03, Visit F 3.66 df 64, 10603 $p < .0001$, Treatment X Visit NS) and the blinded centralized raters (Treatment F 4.56 df 1, 270 p 0.034, Visit F 5.92 df 9, 1537 $p < .0001$, Treatment X Visit F 2.28 df 9, 1537 p 0.015).

Discussion: The PROACTIVE study is distinctive among recent studies that compared LAI RISP to oral antipsychotics in its length and use of both on-site and blinded, centralized Master Raters to assess psychopathology. In general, these other studies have reported similar results to ours; no significant differences in relapse or rehospitalization. In the recently reported study by Rosenheck (2010) rehospitalization by 24 months was approximately 50%. In our study at 30 months the percent is less than 40. In recent studies comparing LAI to oral antipsychotics medications the frequency of contact has been uniform across treatment groups; this frequency may differ from usual care and may impact medication adherence and outcome. Our study differs from others in finding significantly greater improvement in psychotic symptoms over time among subjects who receive LAI RISP than oral medication. Future analyses will examine relapse over the full course of study treatment, other symptoms and social functioning over time to evaluate whether reduction in psychosis translates into better long term functional outcomes.

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59. The Effects of Prolonged Administration of an Alpha7 Nicotinic Cholinergic Agonist Intervention on Neurocognitive Function in Schizophrenia

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Background: Neurocognitive impairment is a core symptom of schizophrenia that impedes recovery and leads to chronic disability. Despite treatment with antipsychotic medications, patients experience residual cognitive deficits, especially in attention and memory. Therefore, cognitive symptoms have become a primary

target of treatment development in schizophrenia. Converging evidence suggests that the alpha7 nicotinic acetylcholine receptor (α_7 nAChR) is a potential target for treatment of cognitive symptoms in schizophrenia. Deficient transduction of the cholinergic signal by the α_7 nAChR is implicated in schizophrenia. Moreover, short-term interventions that stimulate the α_7 nAChR improve psychophysiogical functioning (e.g., P50 evoked response) in patients with schizophrenia. Here we present the findings of a proof-of-concept study to test the prolonged effects of a novel α_7 nAChR agonist intervention strategy on attention and memory in schizophrenia. The combination of galantamine, a positive allosteric modulator (PAM) of nAChRs and CDP-choline, a dietary precursor of choline, which is a selective and potent full alpha7 nAChR agonist, was used to increase α_7 nAChR functioning in negative-symptom patients with schizophrenia.

Methods: This 16-week double-blind, Randomized Clinical Trial (RCT), examined the efficacy, tolerability and safety of the daily administration of the combination of 2 gram CDP-choline and 24 mg galantamine (Galantamine/CDP-choline) versus Placebos added to stable 2nd generation antipsychotic regimens in clinically stable patients who met DSM-IV criteria for schizophrenia or schizoaffective disorder. Patients also had residual negative symptoms as measured by the Positive and Negative Syndrome Scale (PANSS). Tests from the MATRICS Consensus Cognitive Battery (MCCB) were selected to assess the domains of attention (CPT-IP), working memory (Letter-Number Span), verbal memory (Hopkins Verbal Learning Test-Revised), and processing speed (symbol coding). The cognitive battery was administered at baseline, week 8, and week 16.

Results: We randomized 44 participants to treatment; 43 received study medications: 19 (17M; 2F) in the Galantamine/CDP-choline group and 24 (22M; 2F) in the Placebo group. Rate of completion of the entire 16-week trial was nearly equivalent for the two groups: 78.95 % in the Galantamine/CDP-choline group and 79.17% in the Placebo group. There was no statistical difference in baseline characteristics between the two groups in age, education, premorbid IQ, racial and ethnic composition, age of first hospitalization, PANSS scores, or neurocognitive test scores, all P values $>.05$. Premorbid IQ, measured by the revised National Adult Reading Test was in the average range (Galantamine/CDP-choline group: $M = 100.3$, $SD = 9.52$; Placebo group: $M = 102.8$, $SD = 7.6$), but age was on average older than that seen in many studies (Galantamine/CDP-choline group: $M = 54.37$, $SD = 8.50$; Placebo group: $M = 52.38$, $SD = 11.04$). A 2 x 3 (Group x Time) mixed effects model was used to analyze neurocognitive data. The treatment effect is determined by a statistically significant Group by Time interaction. Individual analyses were performed for each measure. Overall, the interaction term did not reach significance for the measures of attention, working memory, verbal memory, or processing speed, all P values $> .05$. The Time effect was significant for the Letter-Number Span ($P < .04$) and the Symbol Coding score ($P < .001$), suggesting practice effects for these tests.

Discussion: This proof-of-concept study tested the effects of a 16-week administration of combined treatment of galantamine and CDP-choline to explore the long-term effects of an α_7 nAChR agonist intervention on neurocognitive deficits in schizophrenia. The cognitive domains of attention and memory were selected because we expected these domains would be sensitive to the effects of α_7 nAChR stimulation based on prior research on nicotinic receptor function and cognition. It is possible that we did not observe an effect of the combination treatment on neurocognitive function because the study was small and therefore underpowered or because the subjects of the study were older and had been ill for many years. It is also possible that the neuropsychological functions assessed by the selected tests lacked the sensitivity to detect a beneficial effect of the α_7 nAChR agonist intervention in schizophrenia subjects. Further research is needed to gain a better understanding of the cognitive and neural processes that are enhanced by stimulation of the α_7 nAChR in this population.

Disclosure: B. Schwartz: None. **S. Deutsch:** Part 1: Astra Zeneca, Merck, OrthoMcNeil Janssen, Part 2: None. **N. Schooler:** Part 1: Astra Zeneca, Bristol Meyers Squibb, Eli Lilly & Company, Hoffman LaRoche, H Lundbeck, Merck, Johnson and Johnson, OrthoMcNeil Janssen, Pfizer, Shire, Part 4: Astra Zeneca Bristol Meyers Squibb Eli Lilly and Company H Lundbeck OrthoMcNeil Janssen Pfizer, Inc. **S. Rosse:** None. **C. Brown:** None. **R. Rosse:** None.

60. A 6-week Randomized, Double-Blind, Placebo-Controlled, Comparator Referenced, Multicenter Trial of Vabicaserin in Subjects with Acute Exacerbation of Schizophrenia

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Background: Vabicaserin is a potent 5-HT_{2C} receptor full agonist that demonstrates *in vitro* functional selectivity for the 5-HT_{2C} over 5-HT_{2A} and 5-HT_{2B}. Vabicaserin is effective in several rodent models that are predictive of antipsychotic activity. Vabicaserin treatment in rats decreases nucleus accumbens extracellular dopamine (DA) levels, without affecting striatal DA, indicating mesolimbic selectivity. This profile is consistent with potential efficacy for the treatment of the positive symptoms of schizophrenia with decreased potential for extrapyramidal side effects. Moreover, preclinical studies suggest the potential for rapid onset of action and improvement in cognitive impairment and negative symptoms associated with schizophrenia. Agonism at the 5-HT_{2C} receptor is known to result in decreased food intake and body weight, suggesting a favorable side-effect profile over the atypical antipsychotics.

Based upon these data, vabicaserin is being developed for the treatment of schizophrenia. The study described here was designed to examine the efficacy, safety and tolerability of vabicaserin in adult subjects with acute exacerbation of schizophrenia.

Methods: Vabicaserin was evaluated in a randomized, double-blind, placebo-controlled and olanzapine-referenced phase 2a study. Hospitalized subjects with exacerbation of schizophrenia were selected and randomized into one of the four treatment arms: vabicaserin 200 mg/day or 400 mg/day, olanzapine 15 mg/day or placebo for a 6-week treatment. Blinded independent central raters performed the PANSS and CGI-S assessment via video-conferencing at screening, baseline and each of the 6 weekly post-baseline visits. Site raters performed the BPRS and CGI-I. Central rated PANSS Positive Score (PPS) was the primary endpoint.

Results: 313 subjects (73% male and 27% female) were randomized and included in the safety analysis with 289 subjects ($n = 77$ in vabicaserin 200 mg/day group, $n = 70$ in 400 mg/day, $n = 71$ in olanzapine and $n = 71$ in placebo) contributing to the mITT population for efficacy analysis. Vabicaserin was well tolerated and no major safety concerns were identified. The most common adverse events were GI related and with a slightly higher rate in female subjects. While olanzapine treatment resulted in weight gain, no significant changes in weight were observed for the vabicaserin treatment ($p < .001$). ANCOVAs (LOCF; mITT) were conducted on the primary endpoint, PSS, and secondary endpoints, PANSS negative, PANSS Total, CGI-S and BPRS. The CGI-I was analyzed using a CMH test. For PSS, vabicaserin 200 mg/day group showed a significant improvement at week 6 (4.2 pt vs. 1.9 pt, $p = 0.03$) compared to placebo. A non-significant decrease versus placebo in PSS score was observed for the 400 mg/day group at week 6 (2.1pt vs. 1.9 pt). The olanzapine group improved significantly compared to placebo (5 pt vs 1.9 pt). For

PANSS negative, the improvements over baseline were significant for both vabicaserin (0.9 pt and 1.1 pt) and olanzapine (2 pt) while placebo was 2 pt worse. For PANSS Total, improvement in the 200 mg/day group was significantly greater than placebo (11.3 pt vs 2.7 pt, $p = 0.011$), whereas the 400 mg/day group showed only a trend of improvement (8.6 pt vs 2.7 pt, $p = 0.085$). The improvement in the olanzapine group was significant over placebo (11.6 pt vs 2.7 pt, $p < 0.001$). For BPRS, no significant improvement versus placebo was observed for either treatment group (200 mg/day: 5.0 pt vs 3.9 pt; 400 mg/day: 5.3 pt vs 3.9 pt). For both CGI-I and CGI-S, significant differences from placebo were observed for vabicaserin 200 mg/day and olanzapine 15 mg/day but not 400 mg/day.
Discussion: In this study, both vabicaserin doses were well tolerated with no significant safety signals detected. Olanzapine treatment resulted in significant weight gain while vabicaserin treatments were weight neutral. Vabicaserin treatment demonstrated efficacy on the primary and all the secondary endpoints at 200 mg/day, but not at 400 mg/day which showed a trend for efficacy signals. Olanzapine treatment was superior to placebo. Overall, these results support the safety and tolerability of vabicaserin in the target population. The 200 mg/day vabicaserin dose group achieved proof of concept using central ratings. Further study is warranted to confirm the efficacy and define the efficacious dose/doses.
Disclosure: J. Shen: Part 1: Pfizer employee, Part 2: As a full time Pfizer employee, I received salaries, bonus, Pfizer stock options and stock awards annually, Part 5: Pfizer Inc. **Y. Zhao:** Part 1: Currently a Pfizer employee, Part 2: Pfizer paid full salary, Part 5: Pfizer. **S. Rosenzweig-Lipson:** Part 1: Former employee of Wyeth/Pfizer; Consultant to AgeneBio, Shire (on unrelated projects), Part 2: Wyeth/Pfizer - Paid Salary AgeneBio - $70,000 (to date). **D. Popp:** Part 1: a full time employee of MedAvante, Part 2: a full time employee of MedAvante, Part 3: a full time employee of MedAvante, Part 5: MedAvante Inc. **J. Williams:** Part 1: MedAvante Inc., Part 2: MedAvante Inc., Part 3: MedAvante Inc., Part 5: MedAvante Inc. **E. Giller:** Part 1: MedAvante Inc. Novartis, Part 2: MedAvante Inc. Novartis. **M. Detke:** Part 1: MedAvante Inc., Part 2: MedAvante Inc., Part 3: MedAvante Inc., Part 5: MedAvante Inc. **J. Kane:** Part 1: Dr. Kane has received honorarium for consulting, participation in advisory boards or lecturing from Amgen, BMS, Intracellular Therapeutics, Janssen, Johnson & Johnson, Lilly, Lundbeck, Merck, Novartis, Otsuka, Pfizer, Pierre Fabre, Proteus, Shire, Sunovion, Targacept, and is a shareholder of MedAvante, Part 2: Dr. Kane has received more than $10,000 per year from: Lilly, Merck, Otsuka., Part 3: No single company provides more than 5% of his annual income.

61. Early Reduction in PANSS-T Score predicts Later Response to Iloperidone Therapy: Results from a Pooled Analysis

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Background: Several studies have shown that early reduction in psychotic symptoms after initiation of antipsychotic treatment predicts later response in patients with schizophrenia. We sought to evaluate this relationship with the atypical antipsychotic iloperidone.
Methods: Data were pooled from 4 double-blind, placebo-controlled, 4–6 week trials evaluating iloperidone in adults with schizophrenia. A 10% or 20% reduction (improvement) in Positive and Negative Syndrome Scale Total (PANSS-T) score at Week 2 was used to assess later response to treatment.
Results: 428 Out of 909 patients met the threshold of a 10% reduction in PANSS-T at Week 2. Mean changes from baseline for PANSS-T scores in these patients at Week 4 were −23.0 for iloperidone 4–8 mg, −26.6 for iloperidone 10–16 mg, and −23.2 for iloperidone 20–24 mg, and −24.1, −29.1, and −27.6, respectively, at Week 6. 217 Patients met the threshold of a 20% reduction in PANSS-T scores at Week 2. Mean changes from baseline for PANSS-T scores among these patients at Week 4 were −29.4 for iloperidone 4–8 mg, −31.1 for iloperidone 10–16 mg, and −30.3 for iloperidone 20–24 mg, and −32.1, −33.0, and −32.8, respectively, at Week 6. Patients who did not meet early threshold criteria had less robust end-of-study reductions in PANSS-T score.
Discussion: Early reduction in PANSS-T score during the first 2 weeks after initiation of iloperidone treatment predicts more robust end-of-study reduction in psychotic symptoms. This general pattern is similar to that seen with other antipsychotic agents.
Disclosure: S. Stahl: Part 1: Advent, Alkermes, Allergan, Allergan, Arena, Arbor Scientia, Astra Zeneca, Biomarin, Biovail, Boehringer Ingelheim, BristolMyers Squibb, Cenerex, Covance, Cypress Bioscience, Dey, Dianippon Sumitomo, Eisai, Eli Lilly, Forest, GenoMind, GlaxoSmith Kline, Janssen, Jazz, LaboPharm, Lunbeck, Marinus, Meiji, Merck, Neuronetics, Neuroscience Education Institute, Novartis, ONO, Orexigen, Otsuka, PamLabs, Pfizer, Pgx Health, Pierre Fabre, Propagate Pharma, Rexahn, Roche, Royalty Pharma, Sanofi, Schering Plough, Sepracor, Servier, Shire, SK Corporation, Soffinova Venturs, Solvay, Sunovion, Valeant, Vanda, Vivus, Wyeth, Part 2: Allergan, Arbor Scientia, Astra Zeneca,Biomarin, Boehringer Ingelheim, Dey, Dianippon Sumitomo, Eisai, Eli Lilly, GenOmind, LaboPharm, LaboPharm, Lundbeck, Meiji, Merck, Neuroscience Education Institute, Novartis, PamLabs, Pfizer, PgX Health, Sanofi, Schering Plough, Servier, Shire, SK Corporation, Sunovion, Vanda, Wyeth, Part 3: N/A, Part 4: Alkermes, Astra Zeneca, Avanir, Biomarin, Boehringer Ingeheim, BristolMyers Squibb, Cenerex, Cephalon, Dainippon Sumitomo, Dey Pharma, Eli Lilly, Forest, GenoMind, Lundbeck, Merck, Neuronetics, Novartis, PamLabs, Pfizer, PgxHealth, Roche, Sanofi, Schering Plough, Sepracor, Servier, Shire, Sunovion, Takeda, Torrent, Trovis, Valeant, Wyeth. **S. Ahmed:** Part 5: Novartis Pharmaceuticals. **X. Meng:** Part 5: Novartis Pharmaceuticals. **L. Warrington:** Part 1: Part 5: Novartis PHarmaceuticals.

62. Adjunctive Treatment with the Selective Glycine Uptake Inhibitor Org 25935 in persistent Negative Symptoms of Schizophrenia: Results from the GIANT trial

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Background: Deficits in glutamatergic neurotransmission have been reported in various symptom domains of schizophrenia, suggesting a therapeutic potential for drugs that could safely enhance NMDA-receptor mediated neurotransmission. The adjunctive use of a selective glycine uptake inhibitor was hypothesized to ameliorate negative and/or cognitive impairment symptoms in subjects with schizophrenia.
Methods: Subjects with persistent predominant negative symptoms in schizophrenia (previously stabilized[3] 3 months on a second generation antipsychotic (SGA)) were eligible for arandomized, placebo-controlled trial to investigate adjunctive treatment with Org 25935, a selective inhibitor of the type 1 glycine transporter, over 12 weeks in a flexible dose design. Adjunctive treatment with Org 25935 was tested at 4-8 mg BID (low Org) and 12-16 mg BID (high Org) and compared with placebo. Primary efficacy outcome parameter was the mean change from baseline (cfb) on the $SANS_{1-22}$ composite score scale. Secondary efficacy endpoints included changes in PANSS total and subscale scores such as the PANSS negative subscore, PANSS Marder negative subscore, or PANSS positive subscore. Secondary endpoints also included depressive symptoms (CDSS scale), global functioning

(GAF scale) and cognitive measures using the CNS-Vital Signs computerized battery (CNS-VS). Responder rates using 2 different responder definitions were assessed post-hoc.
Results: A total of 215 patients were randomized, of which 187 (87%) completed the trial. There was no significant difference between either dose group of adjunctive Org 25935 and placebo in cfb on $SANS_{1-22}$ composite score (mean cfb (SD) points: low Org -13.5 (12.6); high Org:-10.9 (11.9); placebo: -11.2 (11.3). Similarly, no significant differences were found for the majority of secondary outcome parameters such as cfb for PANSS total score, PANSS negative or positive subscale scores, GAF, or the majority of tested cognitive domains. The responder analyses also did not yield consistent results. Org 25935 was generally well tolerated within the tested dose range, with no meaningful effects on EPS symptoms and some reports of transient visual side effects.
Discussion: Org 25935 did not differ significantly from placebo in reducing negative symptoms or improving cognitive functioning when administered as adjunctive treatment to SGA in the 2 chosen dose ranges. In our study population Org 25935 appeared to be well tolerated in the tested dose ranges.
Disclosure: A. Szegedi: Part 1: Merck Sharp & Dohme Corp, Part 2: Stock options with Merck Sharp & Dohme Corp, Part 3: Merck Sharp & Dohme Corp, Part 4: None, Part 5: Merck Sharp & Dohme Corp. **W. Jansen:** Part 1: Merck Sharp & Dohme Corp, Part 2: Merck Sharp & Dohme Corp, Part 3: Merck Sharp & Dohme Corp, Part 4: No Grants, Part 5: Merck Sharp & Dohme Corp. **C. Karson:** Part 1: Merck Sharp & Dohme Corp, Part 2: Merck Sharp & Dohme Corp, Part 3: Merck Sharp & Dohme Corp, Part 4: No Grant, Part 5: Merck Sharp & Dohme Corp. **J. Schipper:** Part 1: Merck Sharp & Dohme Corp, Part 2: Merck Sharp & Dohme Corp, Part 3: Merck Sharp & Dohme Corp, Part 4: No grants, Part 5: Merck Sharp & Dohme Corp. **J. Schoemaker:** Part 1: Merck Sharp & Dohme Corp, Part 2: Merck Sharp & Dohme Corp, Part 3: Merck Sharp & Dohme Corp, Part 4: No grants, Part 5: Merck Sharp & Dohme Corp.

63. Neural Correlates of Emotional Response Inhibition in Obsessive-Compulsive Disorder: Emotional Go/no-go Task Development

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Background: Failure to inhibit recurrent anxiety provoking thoughts is a central symptom of obsessive-compulsive disorder (OCD). Neuroimaging studies have identified the neural correlates of response inhibition deficits in OCD, and show that OCD patients have greater insula activation to disgusting images than healthy controls. OCD patients have not been shown to differ from healthy controls in their neural response to fearful images or faces. Taken together, these findings suggest inhibitory control and disgust processing abnormalities in patients with OCD. However, the emotional modulation of response inhibition deficits in OCD and their neural correlates remain to be elucidated. We adapted an affective response inhibition paradigm, the emotional go/no-go task, to test both disgust and fear related modulation of inhibition in OCD patients with contamination symptoms.
Methods: We administered an emotional go/no-go task to five contamination type OCD patients (thus far) while undergoing fMRI.The emotional go/no-go task measures the ability to inhibit responses to rare non-targets. The paradigm parameters were as follows: six 5 minute blocks with 30 seconds of fixation at start and end (baseline); stimulus onset was 1000 milliseconds (ms); inter-stimulus interval was jittered between 1250 and 1750 ms (mean-1500 ms/block); there were 96 stimuli per block with 72 Go trials (75%) and 24 No-go trials (25%) in total. The trial cues were adapted from the IAPS image collection (Lang 2008). Go cues we neutral (household) images. No-go cues were fear, disgust, and neutral (transport) images. Block order was as follows: i) neutral no-go; ii) disgust no-go; iii) disgust no-go; iv) fear no-go; v) fear no-go; vi) neutral no-go.Commission errors on No-go trials indexed response inhibition.Diagnostic measures administered included the Structured Clinical Interview for the DSM-IV, Yale Brown Obsessive Compulsive Scale, Disgust Scale-Revised, State Trait Anxiety Inventory, Hamilton Depression Rating Scale, Wechsler Abbreviated Scale of Intelligence.
Results: We used event-related analysis with statistical parametric mapping and one-way analyses of variance. Our within-subject factor was image valence (disgust vs. fear vs. neutral) and our dependent measures were percent commission errors on no-go trials and correct no-go brain activation (BOLD signal). Level of significance was set at $p < 0.05$. When exploring neural activation during disgust-related and fear-related response inhibition, in the disgust minus neutral condition, OCD patients had increased bilateral insula and inferior frontal gyrus activation, and left-sided subgenual anterior cingulate activation. In the fear minus neutral condition, OCD patients had increased left-sided insula and inferior frontal gyrus activation, but no amygdala activation. Behaviorally, OCD patients had a higher percentage of commission errors in to fear related stimuli.
Discussion: The results thus far demonstrate the feasibility of the task to assess response inhibition to images with different valences, and show that our emotional go/no-go paradigm is effective. We have clear and distinct activation patterns with only five cases thus far, which indicates that the findings are robust. We are also recruiting more OCD patients and running healthy controls as a comparison group, which is expected to enhance the results even further. The findings thus far indicate that the insula appears to play a central role in the emotional modulation of response inhibition in OCD to both fearful and disgusting images. There was no amygdala activation for fear-related inhibition in OCD patients as opposed to studies in healthy subjects who show robust amygdala activation to fearful images. These results provide important evidence for emotion-specific inhibitory neural pathways in OCD, which may serve as potential targets for novel treatments as well as vulnerability markers for the development of OCD.
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64. Alteration in Neural Response to Emotional Conflict and Conflict Resolution among Behaviorally Inhibited Adults

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Background: About 15% of infants consistently become fearful and distressed when confronted by novelty. When these children then display consistent social reticence in early childhood, they are considered to manifest a "behaviorally inhibited temperament". This temperament is associated with an increased risk for anxiety disorders. Moreover, behaviorally inhibited temperament and social anxiety disorder show similar perturbations in the amygdala and striatum. This raises questions about other biological similarities between behavioral inhibition and clinical anxiety. Recently, Etkin and colleagues (2010) implicated the dorsomedial prefrontal cortex (DMPFC), amygdala and rostral anterior cingulate (rACC) in the detection and regulation of emotional conflict in people with anxiety disorders. The present research extended this work to behavioral inhibition.
Methods: At 4 months of age, 433 subjects were screened for reactivity to novel stimuli, and 153 with extreme levels of high and low reactivity were followed into adulthood. Maternal reports and observations of child behavior at 14, 24, and 48 months of age were used to generate a composite score of behavioral inhibition. Subjects received comprehensive assessments of psychopathology

at ages 15 and 19. At 19 years of age (M = 19.61; SD = 1.62), 37 of these subjects completed the fMRI-emotional conflict task used by Etkin and colleagues. Subjcts included 21 individuals categorized as having high levels of behavioral inhibition and 17 with low levels of behavioral inhibition. In this task, subjects viewed faces with fearful and happy expressions, with the words "happy" or "fear" written across them. Subjects used a button box to identify the emotional expressions of the faces while ignoring the words, which were either congruent or incongruent with the facial expression. Emotional conflict was generated only on incongruent trials, where the word contrasted the facial expression. The conflict between the word and facial expression is expected to activate an anticipatory mechanism, which tends to resolve conflict on a subsequent incongruent trial. Therefore, incongruent trials were categorized as either "high" on conflict resolution, when they were preceded by incongruent trials, or "low" on conflict resolution, when they were preceded by congruent trials. Analyses tested whether subjects with high, relative to low, levels of behavioral inhibition exhibited different responses to trials 1) with versus without emotional conflict, and 2) with high versus low emotional conflict resolution. Neuroimaging results are reported with a statistical threshold of $p < .005$, and extent threshold of 10 voxels unless otherwise noted.
Results: Emotional conflict (incongruent – congruent trials) was associated with elevated activity in DMPFC among subjects with high, compared to low, behavioral inhibition. During the resolution of emotional conflict (incongruent trials preceded by incongruent – congruent trials) there was elevated activity in striatal regions among subjects with high, compared to low, behavioral inhibition. Region of interest analyses targeting rACC revealed diminished activity for subjects with high, compared to low, behavioral inhibition during the resolution of emotional conflict. Only 7 subjects exhibited current psychopathology, whereas 19 exhibited past psychopathology. None of the findings for behavioral inhibition were modulated by psychopathology.
Discussion: This study compared the neural response to emotional conflict in 37 young adults categorized based on their levels of early-childhood behavioral inhibition. Findings revealed a distinct neural-response pattern in these two groups. Relative to prior findings in patients with anxiety disorder, the pattern associated with behavioral inhibition has common and distinct characteristics. Similar to patients with anxiety disorders, behaviorally inhibited subjects exhibited elevated activity in DMPFC during emotional conflict and diminished activity in rACC during the resolution of emotional conflict. Unlike patients with anxiety disorders, behaviorally inhibited subjects did not have perturbations in amygdala activity but rather exhibited elevated levels of striatal activity during the resolution of emotional conflict. These patterns suggest that behavioral inhibitionexhibits both overlapping as well as distinct neural correlates with anxiety disorders for processing conflicting emotional stimuli.
Disclosure: J. Jarcho: None. **N. Fox:** None. **D. Pine:** None. **E. Leibenluft:** None. **T. Shechner:** None. **M. Ernst:** None.

65. Amygdala-Frontal Circuit Function During Threat Perception, Negative Affect Regulation, and Rest Across Anxiety Disorders and Major Depression

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Background: Dysregulated negative affect cuts across anxiety and mood disorders, including generalized social phobia (GSP), post-traumatic stress disorder (PTSD), and major depression (MDD). Aberrant amygdala reactivity is often implicated in these disorders, and at times is related to a non-specific, broad amygdala-frontal cortical network, encompassing anterior cingulate (ACC), medial prefrontal cortex (MPFC), anterior insula (aINS), and ventro/dorsolateral prefrontal cortex (V/DLPFC). No study to date has examined this network across contexts and disorders. Thus, the functional relevance of 'exaggerated' amygdala reactivity related to threat and *how* it manifests across disorders in which negative affect is induced, sustained, or both is still unknown.
Methods: Using functional magnetic resonance imaging (fMRI), we probed amygdala-frontal brain function and connectivity in separate patient cohorts and matched controls across 3 well-validated fMRI tasks in one imaging session. Thus far, three patient groups (GSP, PTSD, and MDD) and 2 control groups (healthy controls, combat-exposed controls) totaling >100 subjects ($\sim n = 20$ per group) have performed a resting state task (intrinsic connectivity at baseline), an emotional face matching task (social threat perception) and/or a cognitive reappraisal task (voluntary regulation of negative emotion). Here we examined amygdala- frontal interactions using two complementary approaches (SPM8, random effects, false discovery corrected): 1) psychophysiological interaction analysis (PPI) to examine task dependent (threat vs. non-threat; reappraise vs. maintain) patterns of amygdala-frontal connectivity; and 2) task-independent amygdala-frontal functional connectivity during resting state. Subject-specific connectivity maps were entered into second-level analyses to examine patterns of connectivity within- and between-groups.
Results: Preliminary analyses have yielded 4 main findings: 1) Large-scale, robust and reproducible task-dependent and task-independent connectivity patterns were observed between the amygdala and primary and higher-order sensory cortices in both patient and control groups; 2) Relative to controls, all three patient groups exhibit *less* connectivity from amygdala to ACC and to DLPFC during threat perception; this pattern is also evident when patients attempt to regulate negative affect; 3) the anxious groups (GSP, PTSD), but not depressed group, showed *more* amygdala-ventral PFC connectivity at rest than controls; 4) Unlike the GSP group, both PTSD and MDD patients have enhanced amygdala-insula connectivity observable at rest. A number of additional analyses are underway: 1) interactions between task and rest networks; 2) delineation of networks based on basolateral (BLA) and centromedial (CMA) amygdala subregions; 3) links between indices of connectivity and symptom severity and clinical dysfunction that are disease specific and non-specific.
Discussion: Confluent abnormalities in dorsal frontal regions (ACC, MPFC, DLPFC) are observed across disorders of dysregulated negative affect, whereas divergent patterns of ventral frontal and paralimbic (insula) dysfunction may differentiate disorders. Patterns of brain network abnormalities that are particular to certain contexts (task-dependent or task-independent) may explain how common forms of psychopathology (i.e., dysregulated negative affect) may have phasic and tonic properties that do, and do not, map onto distinct disorders. Studying a seemingly diagnostically diverse set of patients with the same fMRI probes of amygdala-frontal function may yield a common brain pathway that underlies the dysregulation of negative affect.
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66. Cortisol Response to Awakening in OEF/OIF Combat Veterans with and without PTSD: Relationship to PTSD Symptoms, Treatment Response, and Neural Correlates of Emotional Processing

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Background: The hypothalamic-pituitary-adrenal axis is a stress-responsive neuroendocrine axis which has been long been

implicated in PTSD. While circadian cortisol findings in PTSD have been mixed, recent studies have suggested alterations in morning cortisol response to awakening in trauma-exposed persons. A study of police found negative correlation with PTSD symptoms, and a study of Dutch combat veterans deployed to OEF (Afghanistan) found blunted cortisol response to awakening in veterans with and without PTSD compared to non-deployed controls. Here we examined cortisol response to awakening in two cohorts: OEF/OIF combat veterans seeking treatment for combat PTSD, and OEF/OIF veterans participating in a fMRI neuroimaging study.
Methods: We recruited n = 41 OEF/OIF veterans seeking treatment for combat PTSD and n = 12 healthy OEF/OIF veterans from the VA Ann Arbor, and n = 12 age- and SES-matched healthy non-veteran controls from the community. All participants collected saliva at waking (before getting out of bed) and at 30 min post waking. Salivary cortisol levels were determined using a commercial radioimmunoassay. N = 31 PTSD patients were randomized to either prolonged exposure (PE) or a comparison therapy (Present-centered therapy, PCT), and n = 11 PTSD, n = 10 combat controls, and n = 11 community controls were studied in a cross-sectional neuroimaging experiment in a 3T fMTI environment, using aversive IAPS pictures as an emotional induction.
Results: Total area under the curve (AUCtot) of morning cortisol (wake and wake + 30) was different among healthy controls, and OEF/OIF veterans with and without PTSD (ANOVA $p < .01$), with PTSD patients showing lower cortisol AUCtot than community controls in posthoc ($p < .05$). Morning cortisol were negatively correlated with PTSD symptoms in PTSD patients (total CAPS score $r(41) = -.345$, $p = .02$, CAPS hyperarousal scale, $r(41) = -.378$, $p = .01$), but were not correlated with PTSD symptoms in healthy OEF/OIF veterans. Available data from PTSD patients treated with psychotherapy found PE but not PCT showed a normalization of cortisol awakening response; the PE group showed increase in cortisol waking AUCtot from pre-therapy to post-therapy, and had a higher AUCtot than the PCT group, $F(1,15) = 11.1$, $p = 004$. In fMRI neuroimaging, cortisol waking response (AUC of increase, AUCi) correlated with BOLD response to viewing aversive IAPS pictures (threshold $p < .005$) in the entire fMRI cohort (n = 32) in several medial frontal wall/anterior cingulate (ACC) areas [(6, 39, 24, Z = 3.3), (12, 45, 3, Z = 3.5), (12, 33, 42, Z = 3.5)] as well as posterior cingulate (9, -39, 30, Z = 4.1). Similar correlations were found in both OEF/OIF PTSD (n = 11) and OEF/OIF healthy veterans (n = 10) but not in community controls (n = 11).
Discussion: These data provide further evidence of decreased morning cortisol and response to awakening in recently returning (OEF/OIF) combat veterans. Morning cortisol may have a complex relationship with PTSD. However, these data support a functional relationship both in the correlation between PTSD severity and blunted response to awakening and the normalization of the cortisol dysregulation in Veterans receiving effective treatment for PTSD. fMRI imaging implicates medial wall structures (ACC and PCC) in cortisol response to awakening in trauma-exposed veterans suggesting neural correlates of the HPA axis dysregulation. Implications in regard to potential disease and treatment mechanisms will be discussed.
Disclosure: S. Rauch: None. **A. King:** None. **J. Greco:** None. **E. Smith:** None. **B. Rothbaum:** None. **I. Liberzon:** None.

67. A2 Noradrenergic Neurons induce Fear and Control the Sensitivity to Anxiety-Related Behavioral Responses

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Background: Many neuronal systems have been involved in the regulation of fear and anxiety responses. Brain regions related with these responses such as the amygdala, the bed nucleus of the stria terminalis, the paraventricular nucleus of the hypothalamus, and the nucleus of the solitary tract (NTS) receive noradrenergic (NE) projections. All NE nuclei including the locus coeruleus, the A1 and the A2 nuclei in the brainstem have been reported to project to these regions, but the contribution of each of these NE groups of cells in the stress responses related to fear and anxiety remains unclear. A2 NE neurons in the NTS are good candidates since they send afferents and receive efferents from many of the brain regions related to stress, fear and anxiety. However, the absence of a method to target specific cell types in specific brain regions made it difficult to validate this hypothesis.
Methods: Optogenetic stimulation was used to determine if A2 noradrenergic neurons play an important role in fear and anxiety-related behavioral responses. To specifically target the A2 Ne neurons the adeno associated virus with the double-floxed constructs Ef1a::ChR2-eYFP or its control Ef1a::eYFP were injected in the NTS of TH::Cre transgenic mice. The anatomical projections from A2 NE neurons to stress, fear and anxiety-related brain regions were evaluated. Also, fear and anxiety-related behaviors such as contextual fear conditioning or elevated-plus maze were observed after the optogenetic stimulation A2 NE cells at 10 Hz.
Results: A2 noradrenergic neurons self innervate the NTS, and also send dense projections to the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis and central nucleus of the amygdala. Also, optogenetic stimulation of A2 NE neurons significantly reduces the locomotor activity of animals in a frequency-response manner, but more interestingly, mice expressing ChR2 and optogenetically stimulated in a specific context showed a significant increase in freezing when re-exposed to the same context without stimulation compared to the YFP controls. In addition, ChR2 mice chronically stimulated for 10 days showed a significant decrease in the time spent in the open arms in the elevated-plus maze.
Discussion: These results suggest that A2 NE neurons are sufficient to induce associative learning of the Pavlovian fear conditioning model, and that their repeated activation increases the sensitivity to anxiety-related responses.
Disclosure: C. Tourino: None. **L. de Lecea:** None.

68. Attention Bias Variability and Relations to Symptoms in PTSD

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Background: Posttraumatic stress disorder (PTSD) is a debilitating anxiety disorder that often follows exposure to an overwhelming traumatic event and is characterized by pathologically re-experiencing the trauma, physiological hyperarousal (often including hypervigilance for threat), and avoidance of anything that reminds one of the trauma. Cognitive theories suggest that early, automatic and preconscious information-processing biases play a central role in the etiology and maintenance of anxiety disorders. In line with this theory, attention biases toward threatening or otherwise negatively-valenced information have been observed in several anxiety disorder populations (e.g. social phobia, generalized anxiety disorder). However, very few studies have investigated attention biases in clinical PTSD populations, and the results have been inconsistent across samples and different attention-bias paradigms (e.g. Emotional Stroop task, visual search task), with some studies reporting bias away from threat (avoidance) and others reporting bias towards threat (hypervigilance).
Methods: The current study used the dot-probe task to investigate the characteristics of attention biases (e.g. magnitude and direction

of attention bias, attention bias variability) for threat-related information among symptomatic, non-medicated PTSD (n = 30), trauma-exposed but non-PTSD trauma control (TC, n = 10), and healthy control (HC, n = 30) groups. The dot probe task is a widely used computerized task designed to assess attention biases toward or away from stimuli with certain emotional characteristics (e.g. toward or away from threatening stimuli, such as threat-related words).

Results: Using the dot-probe paradigm, this study found no significant attention biases toward or away from threat-related information in any of the groups, and attention bias was not significantly correlated with measures of depression, anxiety or PTSD symptoms. However, analyses on subjects' within-session variability in bias scores indicated that the PTSD group had significantly greater variability of their attention biases throughout the assessment compared to the TC and HC groups (one way ANOVA, $p < 0.001$), and attention bias variability was significantly and positively correlated with PTSD symptoms (CAPS) in PTSD and TC groups (Pearson's Correlation, r = 0.486, $p < 0.001$). Attention bias variability was also significantly and positively correlated with anxiety (HAM-A, STAI) and depression (HAM-D, BDI-II) severity (Pearson's Correlation, $p < 0.001$). Hierarchical regression analyses indicated that attention bias variability significantly predicted PTSD severity (CAPS score) over and above PTSD diagnosis ($p < 0.001$).

Discussion: As the first study to utilize the dot-probe task to investigate attention patterns in a non-medicated, symptomatic PTSD sample with comparison of non-symptomatic trauma and healthy controls, this study found that the PTSD group showed a significantly more variable attention bias than TC and HC groups, which may underlie the inconsistent reports of both negative and positive biases shown in previous PTSD studies. The attention bias variability could be a prominent marker for the psychopathological processes underlying the hypervigilence and avoidance that are characteristic for PTSD.

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69. Neural Response to Peer Evaluation In Adolescents At Risk For Social Anxiety Disorder Due to Childhood Behavioral Inhibition

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Background: Many adolescents place heightened importance on how they are evaluated by peers; however, excessive focus on peer evaluation can be maladaptive. Anxiety disorders are also common in adolescence, including social anxiety disorder (SAD), a fear of negative evaluation from others and misperceptions of innocuous social events as threatening. Behavioral inhibition (BI) is a temperament identifiable in early childhood that is characterized by withdrawal and wariness of novel and social situations, and conveys risk for the emergence of SAD. Although BI and SAD share phenomenological features (e.g., desiring peer interaction but wary of social interchange), neuropsychological markers linking childhood BI to adolescent SAD are unclear. Initial clues come from evidence indicating amygdala hyperactivation in adolescents with SAD while anticipating and receiving peer evaluation, but this has not yet been examined in adolescents characterized by childhood BI. Furthermore, adolescents with anxiety and those with childhood BI show common patterns of striatal hyperactivation during non-social reward processing. Thus, examining the neural circuitry underlying both reward and threat processing in adolescents with childhood BI during peer evaluation can further elucidate pathophysiological mechanisms of childhood BI as a risk for adolescent SAD. To do so, we examined whether photographs of smiling peers viewed in threatening and rewarding social contexts atypically engaged the amygdala and striatum in adolescents characterized by childhood BI.

Methods: Participants were adolescents assessed through infancy and childhood on temperament and social behavior. Adolescents were characterized as either high in BI (n = 17) or low in BI (non-BI; n = 22) using a composite of measures collected at ages 14 and 24 months and 4 and 7 years, including maternal ratings of shyness and observational measures of social reticence. As adolescents (ages 15-17 years), participants completed a functional neuroimaging task called the "Chatroom," in which they believed that they would interact online with a peer post-scan. Before scanning, participants sorted photographs of unknown peers with smiling, happy expressions into groups of those they select or reject for an online chat, and indicated reasons for these decisions. During functional neuroimaging scanning, participants rated how much interest they thought each peer had in chatting with them. Participants then received pre-determined positive (acceptance) or negative (rejection) feedback from each peer. Regions of interest included the amygdala and striatum (e.g., caudate, nucleus accumbens, putamen).

Results: As expected, BI and non-BI adolescents reported that selected vs. unselected peers would be more interested in interacting with them, $F(1,36) = 46.3$, $p < .001$. BI vs. non-BI adolescents indicated that unselected peers were grouped as such because they looked unfriendly ($p < .05$) and did not seem like fun ($p < .05$). Neuroimaging results indicated greater amygdala activation in BI vs. non-BI adolescents when anticipating evaluation from selected vs. unselected peers, $t(36) = 3.02$, $p < .01$, peak centered at $x = -17$, $y = -7$, $z = -12$. In response to actual peer acceptance vs. rejection, reduced caudate response was found in BI vs. non-BI adolescents ($p < .005$), specifically from selected vs. unselected peers.

Discussion: Adolescents characterized early in childhood with BI showed amygdala hyperactivation to anticipation of evaluation from desired peers and striatal hypoactivation in response to actual acceptance from desired peers. Behaviorally inhibited adolescents display reduced motivation for a positive social interaction and, once confronted with such an interaction, they appear not to find it rewarding. Characterizing neural response to potential social evaluation can inform the degree to which an early-life risk factor for SAD and an actual SAD diagnosis have common or distinct neurobiological underpinnings. Such data are essential for advancing the assessment and prevention of adolescent SAD.

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70. Functional Connectivity of Response Control in ADHD and Pediatric Bipolar Disorder with and without ADHD

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Background: Attention deficit-hyperactivity disorder (ADHD) and pediatric bipolar disorder (PBD) are two developmental syndromes with high comorbidity rates and shared symptoms of inattention, impulsivity and hyperactivity (Galanter and Leibenluft, 2008; Pavuluri and Passarotti, 2008; Passarotti and Pavuluri, 2011). Initial evidence has shown that in spite of similar attention deficits ADHD exhibits more severe prefrontal dysfunction relative to PBD during a response inhibition task (Passarotti *et al.*, 2010). In this study, Independent Component Analysis (ICA) methods

(Calhoun *et al.*, 2008) were used to further examine the neural bases of impulsivity in distributed brain networks engaged during execution or inhibition of a pre-potent motor response in adolescents with PBD and adolescents with ADHD relative to healthy controls (HC). We included a PBD group with ADHD (PBD + ADHD) to further examine the effects of ADHD on PBD psychopathology.

Methods: Participants. Thirthy-one adolescents with PBD, 24 adolescents with PBD + ADHD, 22 adolescents with ADHD and 33 HC (mean age 13.78 ± 2.4), matched for demographic variables. Task and fMRI session. Participants underwent a 7-min event-related fMRI stop signal task (SST), examining the ability to inhibit a prepotent motor response 'already on the way'. fMRI data acquisitions and ICA Data Analyses. We performed gradient-echo echo-planar imaging (EPI) using a 3 Tesla G.E. scanner. Standard structural images were also obtained. ICA methodology identified spatially independent and temporally coherent networks underlying functional brain networks for this task.

Results: No significant group differences were found in behavioral performance. ICA analyses revealed two functional brain networks that were related to the task and exhibited group differences in functional connectivity. *Attentional Response Control Network*. Within an attentional response control network (Zhang and Li, 2011) PBD relative to ADHD exhibited greater functional connectivity in right medial frontal gyrus and insula and reduced connectivity in right inferior parietal lobe and cerebellum. Relative to HC both PBD and ADHD exhibited greater functional connectivity in right VLPFC. Moreover, relative to HC PBD exhibited greater connectivity in parahippocampal gyrus and left caudate, while ADHD showed greater connectivity in right inferior parietal lobule, and decreased connectivity in left temporo-parietal regions. Finally, the PBD + ADHD group showed a more severe PBD-like pattern of over-engagement in emotional regions such as the insula, and a less severe ADHD-like pattern of under-engagement in parietal and midcingulate attentional regions (connectivity in these regions was decreased relative to PBD and HC, but increased relative to ADHD). *Motor Control Network*. Within a fronto-striatal -cerebellar network relative to HC ADHD showed greater connectivity in bilateral cerebellum and decreased connectivity in left precuneus and subgenual ACC. Relative to ADHD the PBD group exhibited increased connectivity in left DLPFC and left putamen, and decreased connectivity in left amygdala and right cerebellum. Relative to HC PBD exhibited greater functional connectivity in left insula and decreased connectivity in left amygdala. For the PBD + ADHD group functional connectivity was reduced in limbic regions relative to the other three groups, while it was increased in right putamen relative to PBD and ADHD, and in left DLPFC and right insula relative to ADHD.

Discussion: The present findings suggest that within an attentional control network, relative to HC both PBD and ADHD groups exhibit greater engagement in right VLPFC, possibly related to greater sensitivity to prepotent motor responses. The PBD group also showed increased connectivity in emotional evaluation regions possibly due to greater emotional engagement during attentional control, and reduced cerebellar involvement relative to ADHD. Furthermore, within a motor control network, the PBD and PBD + ADHD groups showed reduced functional connectivity in limbic regions relative to ADHD and HC, suggesting poor affective regulation during motor control, while functional connectivity was increased in fronto-striatal regions relative to ADHD, suggesting less severe motor control deficits. Relative to PBD and HC the ADHD group showed increased cerebellar engagement, possibly related to hyperactivity and poor motor control. These findings, while preliminary, shed light on the different phenotypy of impulsivity in ADHD and PBD and are in line with the view of greater cognitive impulsivity in ADHD and more severe emotional impulsivity in PBD (Passarotti and Pavuluri, 2011).

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71. Fronto-Opercular Control Circuits Mediate the Effect of Methylphenidate on Reaction Time Variability

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Background: Reaction time variability (RTV), within-subject fluctuation of reaction times across trials of a single task episode, is a highly sensitive measure of attention functioning. RTV is elevated in psychiatric disorders characterized by attention dysregulation such as ADHD, and the processes that are thought to generate elevated RTV (e.g., diminished cognitive control, dysregulated endogenous rhythms), are likely to be central to the pathophysiology of the disorder. Psychostimulants decrease RTV in both healthy individuals and individuals with ADHD. However, the brain basis of RTV as well as psychostimulant-driven attenuation of RTV are poorly understood.

Methods: In a double-blind, randomized, crossover design, 26 healthy stimulant-naïve participants received either placebo (PBO) or 40 mg oral methylphenidate (MPD) 60 minutes prior to fMRI scanning (i.e., each subject was scanned twice). During each scan, all participants performed two runs (trials = 100 per run) of an event-related multisource interference task (MSIT), which robustly engages fronto-opercular cognitive control circuits. RTs for each subject, scan, and run were fit using an ex-Gaussian distribution parameterized by μ (mean), σ reflecting Guassian standard deviation ('attentional flux'), and σ reflecting long RT trials ('lapses of attention'). BOLD-sensitive scans were analyzed in a random effects analysis implemented in SPM8. Two neuroimaging analyses were performed: 1) a 'condition analysis' examined mean activation across task conditions between PBO and MPD; 2) a 'trial-by-trial analysis' used deviation of reaction time from the mean as a trial-by-trial parametric regressor in a finite impulse response (FIR) model. A 15 TR (30 second) time window spanning 10 seconds pre- and 20 seconds post-stimulus was examined to identify brain regions that predict trial-to-trial variation in RT (see Weissman *et al.*, 2006).

Results: Behavioral results: Compared to PBO, MPD significantly reduced mean RT, as well as RTV [σ ($p < 0.05$) and τ ($p < 0.1$)]. The τ component of RTV increased during run 2 compared to run 1 of PBO ($p < 0.05$), which prior research attributes to the effects of fatigue. However, this τ increase was absent during the MPD session (PBO > MPD, $p < 0.05$). Scores for the Conners' Adult ADHD Rating Scale - Inattention Subscale (CAARS-I, higher scores = greater inattention) significantly predicted RTV (both σ and τ) during PBO ($ps < 0.05$), but were uncorrelated with RTV during MPD.

Neuroimaging results: *Condition analysis*: In whole-brain analysis, during PBO, both congruent and incongruent trials robustly activated fronto-opercular control regions including right and left inferior frontal gyrus/insula ([36, 23, 7], z = 5.91) and dorsal medial prefrontal cortex extending to pre-supplementary motor cortex ([-6, -1, 55], z = 9.94). During MPD, activation in this fronto-opercular network was significantly enhanced ($p < 0.05$). Higher CAARS-I scores predicted diminished activation in the fronto-opercular network during PBO. However, individuals with higher CAARS-I scores showed greater enhancement in activation in fronto-opercular regions during MPD scans relative to their PBO scans.

Trial-by-trial analysis: Starting 4-6 seconds prior to stimulus onset, fronto-opercular regions exhibited elevated activity during MPD compared to PBO. In the 2-6 second post-stimulus interval, activity in fronto-polar cortex predicted shorter RT during MPD, but not during PBO.

Discussion: This study used a combination of condition analysis and trial-by-trial analysis to establish a critical role for fronto-opercular control circuits in regulating RTV, with MPD-driven enhancement of activation in these circuits associated with RTV attenuation. MPD effects were heightened in individuals with high levels of trait inattention, suggesting that baseline level of attention functioning moderates MPD's effects. These results shed important new light on the brain pathways by which psychostimulants reduce RTV. They also add the growing body of evidence that RTV (and the mechanisms that generate it) play a central in both the pathophysiology of attention dysfunction and the mechanisms by which psychostimulants enhance attention functioning.
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72. Cognitive and Clinical Outcomes Associated with Cannabis Use in Patients with Psychotic Disorders

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Background: Cannabis is the most widely used illicit substance in Western countries and particularly high prevalences have been shown in patients with major psychotic disorders. Furthermore, a growing body of evidence suggests a consistent association between its use and psychotic symptoms. 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.
Moreover, several studies have suggested that cannabis use in patients with psychotic disorders may be associated with variation in cognitive function, clinical presentation and course of illness, the effects have been inconsistent. The objective of the present study was to compare clinical and neuropsychological measures in individuals diagnosed with a major psychotic disorder with a history of cannabis use disorder (CUD) versus individuals with psychotic disorder without a history of CUD.
Methods: We ascertained a large cohort (N = 594) of patients diagnosed with a major psychotic disorder schizophrenia, schizoaffective and bipolar disorder with psychotic features) with either no history of a CUD (CUD−; N = 356) or a history of CUD (CUD +; N = 186). The groups were initially compared on key demographic variables including sex, race, age, duration of illness, parental socioeconomic status, premorbid IQ, education level and global assessment of functioning. After covarying for any observed differences in demographic variables, we compared groups on lifetime measures of psychotic symptoms as well as a brief battery of neurocognitive tests.
Results: Compared to the CUD− group the CUD + group had significantly better GAF scores but less years of education. After correcting for these differences the CUD + group demonstrated significantly better performance on measures of processing speed (Trail Making Tests A and B), working memory (Digits Backward), verbal fluency (letter and animals) and verbal learning (California Verbal Learning Test).
Discussion: Collectively, these findings suggest that patients with major psychotic disorders with comorbid CUD may represent a higher functioning subgroup of SZ. Future prospective studies are needed to elucidate the nature of this relationship.
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73. Executive Function in Alcohol-Dependent Adults With and Without Comorbid Bipolar Disorder: Performance on the Color-Word Interference, Alcohol, and Emotion Stroop Task

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Background: Alcohol dependence is extremely common in individuals with bipolar disorder and is often associated with a severe course of affective illness, poor role functioning, and low quality of life. Though impairments of neurocognitive function have been well documented independently in patients with either alcohol dependence or bipolar disorder, the extent of cognitive impairment in adults with both illnesses has received limited research focused primarily on comparisons of cognitive performance in bipolar subjects with and without alcohol dependence. The current study evaluates executive functioning in alcohol-dependent subjects with and without comorbid bipolar disorder using color-word interference, alcohol-related, and emotion-related variants of the Stroop task.
Methods: Sixty adults aged 18-65 were screened for substance use and affective disorders using the Structured Clinical Interview for DSM-IV and for other Axis I psychiatric disorders using the Mini International Neuropsychiatric Interview. Those who met lifetime DSM-IV criteria for alcohol dependence (N = 44) were eligible for testing of performance on a neurocognitive battery including the color-word interference, alcohol, and emotion Stroop tasks. Of this sample, a subset of subjects (N = 26) also met criteria for bipolar I disorder or bipolar II disorder and were maintained on stable doses of mood stabilizing medications including lithium, lamotrigine, valproic acid, or second-generation antipsychotic agents. Subjects in each group taking the antidypsotropic medications disulfiram, naltrexone, acamprosate, or topiramate were excluded. Stroop task performance was evaluated using computer-generated neutral and active (color-incongruent, alcohol-related, or negative/positive emotion-related) stimulus presentation coupled with real-time recording of response errors and reaction times in milliseconds via an interfaced response box with color-coded buttons. Color-word interference and alcohol Stroop trials consisted of successive presentations of 48 neutral and active stimuli for each test modality. Emotion Stroop trials consisted of successive presentations of 64 neutral and active stimuli containing negative and positive emotionally salient words, respectively. Results were analyzed separately for each Stroop task modality using ANOVA and general linear statistical models.
Results: No main effect of group or group X stimulus interaction were evident for error rates on the color-word interference, alcohol, or emotion Stroop task. Significant main effects of active vs. neutral stimulus presentation on reaction times were observed in the sample as a whole for the color-word interference ($p < .001$), negative emotion ($p < .01$), and positive emotion ($p < .005$), but not the alcohol ($p = .27$) Stroop task. However, a marginally significant group X stimulus interaction was evident in reaction times recorded on the alcohol Stroop task ($p < .057$); upon presentation of active alcohol-related words, subjects with bipolar disorder + alcohol dependence exhibited greater delays (mean = 53 msec) relative to neutral stimuli than did alcohol-dependent subjects without bipolar disorder (mean = -14 msec). No other group main effects or group X stimulus interactions were evident for the color-word interference or emotion Stroop tasks.
Discussion: In the present sample, subjects with bipolar disorder and co-occurring alcohol dependence appeared to have greater delays in reaction time when presented with alcohol-related word stimuli than did alcohol-dependent subjects without bipolar disorder. These results appeared to be selective for the alcohol Stroop task and did not extend to the color-word interference or emotion Stroop tasks. Because the alcohol Stroop task has been used as a measure of attentional bias for alcohol-related cues, these results may suggest that alcohol-dependent individuals with bipolar disorder may

preferentially attend to alcohol cues relative to their nonbipolar counterparts with alcohol dependence. Future studies are necessary to explore the prognostic and treatment implications of these findings.
Disclosure: B. Tolliver: Part 4: Forest Laboratories, Part 5: Medical University of South Carolina. **D. Brown:** None. **J. Prisciandaro:** None. **K. Brady:** None.

74. From Brain to Behavior - Neural Correlates of Real-World Functioning Difficulties in Patients with Bipolar Disorder: An fMRI Study

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Background: Bipolar disorder is characterized by cognitive impairment including difficulties in attention, memory and executive functioning (Goldberg & Berdick, 2008). Neuroimaging studies have examined the neural correlates of cognitive functioning in bipolar disorder. However, there is a lack of studies investigating the link between cognitive difficulties expressed in daily life and neural abnormalities found in bipolar disorder (Manove & Levy, 2010). In the present study, we examined the relationship between self-reported executive functioning difficulties and brain activation in individuals with bipolar disorder using functional Magnetic Resonance Imaging (fMRI).
Methods: 30 right-handed DSM-IV bipolar-I disorder patients (20 females, mean age = 37.6, SD = 13.0, mean education = 15.5 years, SD = 1.4) completed the Frontal Systems Behavior Rating Scale (FrSBe, Grace & Malloy, 2001), a behavioral measure of executive dysfunction. Patients also completed a variant of the Multi-Source Interference Task (MSIT) while undergoing fMRI. In this task participants were shown three-digit numbers (e.g. 100, 020 or 003) and had to decide which number was different from the two other numbers (e.g. 100; correct answer = 1). A non-interference trial consists of the "different" number in its corresponding position (e.g. 100). An interference trial consists of the "different" number not in its corresponding position (e.g. 221). We conducted regression analyses using FrSBe T-scores of Executive Dysfunction as predictors of the fMRI BOLD contrast interference vs. non interference using Statistical Parametric Mapping software (SPM5, Wellcome Department of Cognitive Neurology).
Results: BP-I patients showed elevated T-scores in Executive dysfunction (M = 67.25, SD = 17.1). Our regression analyses showed positive correlations ($p < .01$) between Executive Dysfunction T-scores and bilateral activations in the dorsolateral prefrontal cortex (DLPFC; Brodmann area [BA 9]), bilateral activations in the inferior temporal lobes in BA 37, ventrolateral prefrontal cortex (BA 11/47) and supplementary motor cortex. We found negative correlations between Executive Dysfunction T-scores and dorsal and rostral anterior cingulate (ACC) activation as well as bilaterally with the dorsomedial prefrontal cortex.
Discussion: Most notably, daily difficulties in executive functioning were associated with an inverse correlation between DLPFC and ACC activations and executive functioning. This result suggests disruption in the crosstalk of the ACC-DLPFC system (Pizzagalli *et al.*, 2011).
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75. Neural Response to Monetary Reward in Depression: A Meta-Analysis

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Background: Neural response to reward is postulated to play a key role in the etiology, development, pathophysiology, and treatment of depression (e.g., Forbes & Dahl, 2005; Hasler, Drevets, Manji, & Charney, 2004). Consistent with conceptual models of diminished positive affect in depression, functional magnetic resonance imaging (fMRI) research on reward function can illustrate the biological underpinnings of this critical aspect of disrupted affect in depression. Several studies have reported that depression is associated with lower striatal response to the anticipation and receipt of rewarding stimuli such as money and happy facial expressions. Given the widespread use of monetary reward fMRI paradigms and the basic nature of money as a reward stimulus, we conducted a meta-analysis of studies of striatal response to monetary reward in depression. Because studies have been conducted with both adolescents and adults, and given that developmental issues have been proposed as important to understanding the role of reward function in depression, we also conducted exploratory comparisons of developmental effects.
Methods: Comprehensive literature searches using 3 citation indices and the search terms "fMRI," "depress*," and "reward*" were conducted to identify appropriate articles in peer-reviewed journals written in English. This search identified 86 potential studies, of

which 5 studies met inclusion criteria. Of these, 4 studies examined striatal response to the anticipation of monetary reward, and 5 studies examined striatal response to reward outcome. Inclusion criteria were the following: fMRI using monetary reward; index of depression diagnosis or symptom severity; results presented in Talairach or Montreal Neurological Institute space to permit converstion and allow comparisons between studies; reporting of sample size and peak voxel location; and sufficient statistics to include in activation likelihood estimation (ALE) and effect-size-based meta-analytic procedures. We included all reported study results, regardless of the specified alpha level for the studies. Two of the included studies reported null findings.

Results: For both reward anticipation and reward outcome, ALE meta-analysis revealed that there were systematic differences across studies between depressed and healthy samples. During reward anticipation, function in a single region in the caudate distinguished depressed and healthy individuals across studies. During reward outcome, 4 clusters—in the ventral striatum, caudate, putamen, and globus pallidus—were identified that differed between depressed and healthy individuals across studies. Using fixed-effects meta-analytic procedures, the observed effect size for striatal reactivity differences between depressed and healthy participants was .53 (confidence interval (CI): .24-.83) for reward anticipation and 1.13 (CI: .83-1.46) for reward outcome. These non-overlapping CIs suggest that effects for depression were larger for reward outcome than for reward anticipation. Exploratory analyses suggested that in both adult and youth samples, depression was associated with low striatal response to reward anticipation and reward outcome. Effect sizes for depression were modestly larger for adults relative to youth, although the number of studies in each developmental group was limited.

Discussion: This meta-analysis is a critical first step in integrating the research findings on affective processing in depression. Even when accounting for studies with null findings, our meta-analysis revealed that striatal function is disrupted across studies, fMRI tasks, measures of depression, and developmental groups. Together, these findings point to the possibility that disrupted reward function in the striatum is a biomarker of depression.

Disclosure: T. Olino: None. **E. Forbes:** None.

76. The 2-Year Course of Cognitive Function and IADLs in Older Adults with Bipolar Disorder

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Background: Bipolar Disorder (BD) is a leading cause of disability worldwide. The disability associated with BD exceeds that of major depressive disorder and approaches that of schizophrenia, with which it shares a similar prevalence in the U.S. population. An important contributor to disability in BD is cognitive impairment. The purpose of this analysis is to characterize the trajectory of cognitive function in a sample of older individuals with BD. An examination of distinct cognitive domains may help both clarify areas of pathology and potential types of interventions. Based on our prior reports, preliminary data, and the existing literature, our main hypothesis was that, compared with a group of mentally healthy individuals of similar age and education, older adults with BD would exhibit greater cognitive decline over two years in the domains of information processing speed and executive function, but not in other domains. Related to our interest in disability, we also explored the relationship between cognitive function and instrumental activities of daily living (IADLs) over time.

Methods: Study subjects: Individuals with BD I or II ($n = 47$) were recruited from outpatient clinics or treatment studies carried out at the University of Pittsburgh. Comparator subjects ("controls") were 22 age- and education-equated subjects with no psychiatric or neurologic history. Comparison subjects were selected to make the groups similar in age, education, and cardiovascular burden. All participants provided written informed-consent, approved by the Institutional Review Board at the University of Pittsburgh.

Inclusion criteria were: age 50 years or older; clinical euthymia for four weeks preceding neuropsychological (NP) assessment with scores of 10 or less on the 17-item Hamilton Rating Scale for Depression-17 item (Ham-D) and 10 or less on the Young Mania Rating Scale (YMRS) at the time of assessment; ability to comprehend and speak English fluently; and corrected visual ability to read newspaper headlines and hearing capacity adequate to respond to a raised conversational voice. Exclusion criteria were: pre-existing history of dementia or neurologic disorder affecting the central nervous system (for example, Parkinson's disease, traumatic brain injury, or multiple sclerosis); electro-convulsive therapy within the past six months; and substance abuse or dependence within the past twelve months.

Measures: We employed a broad-based assessment of cognitive function and IADLs. The NP Evaluation encompassed 21 well-established and validated individual tests measuring multiple cognitive domain organized into a global score and four distinct cognitive domains: Delayed Memory; Information Processing Speed/Executive Function; Language; and Visuomotor. We assessed IADLs using a criterion-referenced, performance-based instrument, the Performance Assessment of Self-Care Skills (PASS). IADLs scores ranged from 0 (complete independence) to 9 (complete dependence) such that higher scores indicated worse performance. Medical illness burden was measured with the Cumulative Illness Ratings Scale-geriatric (CIRS-G). A cardiovascular subscale was composed of CIRS-G items #1 (heart) and #2 (vascular). Statistical Analysis: We employed a repeated-measures mixed-effects linear model with subject as a random effect and time as random and linear, rendering random slope, and included age and education as covariates. We employed non-parametric tests for analysis of IADLs.

Results: As a group, BD participants displayed worse cognitive function than mentally healthy comparators at baseline and over follow-up in all domains and globally ($F = 23.41$, $p < .001$). BD participants displayed worse IADL performance at baseline and over follow-up (Wilcoxon rank-sum exact test $p = 0.02$). Among the BD participants, baseline IADLs were correlated with global cognitive function ($n = 47$, $r_s = -0.45$, $p = .0015$).

Discussion: Over two years of repeated cognitive assessment, we find that older adults with BD perform worse than mentally healthy comparators of similar age, education, and cardiovascular burden. Individuals who start at a lower level of cognitive performance will cross the threshold for expression of clinically significant cognitive impairment and dementia sooner than individuals with greater cognitive reserve. Our report further suggests the need for individuals with BD to focus on preventive measures that may help with preservation of cognitive function, focusing on healthy lifestyle behaviors and addressing treatable medical illnesses, such as hypertension, hypercholesterolemia, and overweight/obesity.

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77. Impaired Fixation to Eyes in Children with Bipolar Disorder or Severe Mood Dysregulation

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Background: Children with bipolar disorder (BD) or severe mood dysregulation (SMD) have deficits in face emotion information processing. The children show low accuracy in labeling emotional expressions and exhibit abnormal amygdala responses to emotional expressions. These behavioral and neural deficits may be associated with abnormal eye-movements during face scanning. Literature suggests that adults and children with autism or schizophrenia are less accurate in labeling emotional expressions, and that their poor labeling performance is associated with reduced fixations on emotionally salient facial features such as eye regions. Thus, we examined whether decreased attention to eye regions in children with BD or SMD may be associated with their face emotion labeling deficits.
Methods: Participants included children with BD (N = 18), or SMD (N = 26), and healthy volunteer (HV) children (N = 19). All groups were matched by age, IQ and gender. Eye movements were measured with an EyeLink II headmounted eye-tracker (SR Research, Mississauga, ON, Canada), and sampled pupil centroid at 500 Hz. Before starting the task, the eye-tracker was calibrated and validated to participants' eyes, and a drift correction was performed every 5 trials during the task. Participants were shown 130 photographs total of four facial expressions (neutral, happy, sad, angry, and fearful). After seeing a face for 2 seconds, participants were given 3 seconds to identify the face emotion. A blank screen was shown for 1 second between trials. Rectangular areas-of-interest (AOIs) were drawn around the eyes, nose, and mouth. Data were analyzed using a repeated-measures analysis of covariance (ANOVA) where group (BD, SMD, HV) was a between-group factor and emotion and AOI were within-group factors.
Results: Across emotions, SMD children showed lower accuracy than BD and HV children whereas BD children tended to show slower reaction time than SMD and HV children. For fixation number, there was a group X AOI X emotion interaction. For angry, fearful, sad and neutral expressions, BD children made fewer fixations to the eye regions than HV children. For fearful faces, SMD children also made fewer fixations to the eye regions than HV children. No between-group differences were found for fixation duration.
Discussion: Abnormal eye-movements such as decreased attention to eye regions among BD and SMD children may mediate their face emotion labeling deficits. Our results may provide important information for future research investigating atypical gaze in relation to brain function and for intervention studies training patients to attend to eyes.
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78. Social Cognitive Performance Profile of Bipolar Disorder and Schizophrenia

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Background: Patients with bipolar disorder exhibit substantial disability that has not been satisfactorily explained by clinical symptoms. Research in schizophrenia has identified social cognition as a key determinant of functional outcome of patients. Considering several intriguing parallels between bipolar disorder and schizophrenia (e.g. treatment response, common cognitive impairments), social cognition may also play a key role in functional impairment in bipolar disorder. However, little is known about social cognition in bipolar disorder. Existing studies on social cognition in bipolar disorder have mainly focused on facial affect recognition, and other areas of social cognition have been largely overlooked. This study aimed to characterize social cognitive performance of patients with bipolar disorder using social cognitive measures across key social cognitive domains.
Methods: Forty-eight clinically stable patients with bipolar disorder, 29 clinically stable patients with schizophrenia, and 23 psychiatrically healthy controls completed a battery of social cognitive measures and the MATRICS Consensus Cognitive Battery (MCCB). Social cognitive measures were divided into two categories: Two measures reflected low-level social cognition including: a) Facial Affect Recognition Task, and b) The Awareness of Social Inference (TASIT) - Lie Scale. Four measures reflected high-level social cognition including a) Empathic Accuracy Task, b) Self-referential Memory Bias task, c) TASIT - Sarcasm Scale, and d) Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) - Managing Emotions.
Results: For the two low-level social cognitive measures, schizophrenia patients showed impaired performance relative to healthy controls and to patients with bipolar disorder, but the latter two groups did not differ from each other. For the four high-level social cognitive measures, we observed a similar pattern, such that schizophrenia patients showed poorer performance compared to patients with bipolar disorder and controls, who did not differ from each other. Exploratory analysis on subgroups of bipolar disorder indicated that patients with a history of psychosis showed poorer performance than patients without such a history only for the four high-level social cognitive measures. Due to small number of patients with bipolar disorder who also had a history of psychosis, it was not possible to perform a rigorous statistical test on this pattern. For non-social cognition, schizophrenia patients showed the most impaired performance and patients with bipolar disorder were intermediate and significantly different from both schizophrenia patients and controls.
Discussion: For both low-level and high-level social cognitive measures, patients with bipolar disorder showed better performance compared to schizophrenia patients. Further, social cognitive performance of patients with bipolar disorder was comparable to that of controls. The current study suggests intact social cognitive performance in bipolar disorder as a whole but exploratory analysis with subgroups of patients with bipolar disorder suggested that patients with a history of psychosis may have different social cognitive performance profile than patients without such a history. It will be informative to examine whether this pattern can be observed with larger subgroups of bipolar disorder based on a history of psychosis. This relatively intact social cognitive performance in bipolar disorder suggests that the way social cognition affects functional outcome may differ between bipolar disorder and schizophrenia.
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79. Developing Translational Neurocognitive Measures Specific and Sensitive to Electroconvulsive Therapy

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Background: Standard neurocognitive measures are typically employed to characterize electroconvulsive therapy (ECT) asso-

ciated cognitive effects in patients with depression. While those measures are useful, they are also limited as they confound reaction time and accuracy data, detect only broadly defined cognitive functions, and are sensitive to depression. Thus, newer cognitive assessment methods are needed that that allow for the precise measurement of reaction time and accuracy, are insensitive to depression, and are able to inform the cognitive component processes impacted by ECT. We developed a battery of five touch-screen, computerized, neurocognitive measures in a nonhuman primate model that assess short-term, long-term, and working memory, and found that the measures were sensitive and specific to ECT. Importantly, the measures were able to assess distinct memory functions and cognitive component processes. In the initial adaptation of the measures for human use in a healthy cohort, we found that performance on the measures was associated with demographic variables of age, education, and estimated IQ. The purpose of this study was to assess if the adapted neurocognitive measures were sensitive to depression and cognitive inefficiency in humans.

Methods: To translate the neurocognitive measures for human use, the test stimuli and parameters used in the basic science model were placed on a custom-modified, touch-screen computer. As the measures are being adapted for use in depressed populations undergoing ECT, we assessed the performance of 21 severely depressed patients after they were washed off psychotropic medications. We compared their performance to two comparator groups, a healthy cohort ($N = 40$) free of psychiatric and medical illnesses, and a cohort with Parkinson's disease ($N = 15$). We chose PD as a comparator group due to its similar subcortical neurocognitive profile as depression. All participants completed the battery of neurocognitive measures (Target Identification, Target Tracking, Target Sequencing, Spatial Configuration, and Serial Target Recognition). Analysis of variance models were computed followed by post-hoc tests to compare and contrast performance between the groups. Statistical significance was defined as a two-sided p-value of less than 0.05.

Results: The three cohorts (healthy (HC), depressed (MDD), PD) were similar with regard to most demographic variables except for age (PD > HC = MDD; $F(2,74) = 21.9$, $p < 0.0001$) and depression severity (MDD > HC = PD; $F(2,73) = 254.8$, $p < 0.0001$). For the Target Identification task, there was a significant difference between groups for accuracy ($F(2,67) = 4.5$, $p = 0.02$), with the PD cohort showing worse performance than the HC and MDD cohorts. There was no difference between groups regarding completion time. On the Target Sequencing task, there was no difference between groups in terms of accuracy, but there was a significant difference with regard to completion time ($F(2,66) = 15.6$, $p < 0.0001$), with the MDD cohort taking longer time to complete the task relative to the healthy and PD cohorts. Accuracy on the Spatial Configuration measure significantly differed between cohorts ($F(2,67) = 4.5$, $p = 0.02$), with the PD cohort showing poorer performance relative to the healthy and MDD cohorts, but there was no difference regarding completion time.

Discussion: The adaption of the basic science neurocognitive measures that assess short-term, long-term, and working memory, was feasible in healthy human subjects and patient populations. Importantly, depression was found to not impact accuracy on the neurocognitive measures, though it did result in relatively lengthier time to complete one of the measures. This is consistent with prior findings showing depression to have a substantive adverse impact on processing speed, and further highlights the advantage of developing neurocognitive measures that separate the variables of accuracy and reaction time. The testing of the PD cohort demonstrated that these adapted neurocognitive measures are sensitive to inefficiencies in neurocognitive performance. Collectively, these data suggest that these translational neurocognitive measures may be of benefit for depressed patients treated with ECT as they may be able to detect adverse cognitive effects related to the treatment without being confounded by the effects of the depressive illness. Future research is warranted to continue the translation of these measure for clinical use by assessing associations with other demographic factors, standard neuropsychologic measures, psychiatric disease, and psychopharmacologic challenges.

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80. Effects of Sertraline on Neurocognition in Outpatients with Major Depressive Disorder

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Background: Disturbances of cognitive and psychomotor functioning are common in patients with depression. How these deficits are affected by antidepressant medication treatments, such as serotonin re-uptake inhibitors (SSRIs) is of interest both for clinical reasons and because of the extensive serotonergic innervations of neocortical and striatal regions known to modulate cognition and behavior.

Methods: Fifty-four midlife outpatients with nonpsychotic major depression completed a 28-day medication free period during which they received supportive clinical management and single blind placebo to ensure the presence of persistent depressive symptoms at the time of baseline testing. Over this lead-in phase, 15 patients experienced an improvement in their clinical symptoms (Hamilton Depression Rating Scale (HAM-D) < 15) and were identified as placebo responders. Twenty-eight demographically and IQ matched healthy individuals were also studied at one time point to characterize the extent of pretreatment deficits. All subjects completed oculomotor tasks designed to evaluate the frontostriatal systems regulating psychomotor functions, working memory (memory guided saccades), and voluntary inhibitory control (antisaccades). Subjects also completed a battery of neuropsychological tests. After baseline testing, patients whose depressive symptoms persisted (HAM-D ratings > 15) were randomly assigned to receive double blind placebo plus supportive counseling ($n = 13$), cognitive behavioral therapy (CBT; $n = 14$), or sertraline plus supportive counseling ($n = 12$; titrated to mean daily dose of 137.5 + /-43.3 mg). After 12-weeks patients underwent repeat testing.

Results: At baseline, patients not identified as placebo responders demonstrated significantly longer antisaccade latencies and reduced smooth pursuit gain compared to healthy individuals. On neuropsychological measures, no deficits were detected relative to controls. At the 12-week follow-up, patients demonstrated improvement on oculomotor measures including a significant reduction of their pretreatment impairments on antisaccades and smooth pursuit gain. Patients treated with sertraline demonstrated the greatest improvement in antisaccade latency, reflecting reduced psychomotor slowing and improved ability to quickly plan and initiate behavior. Improved performance was also observed across some neuropsychological measures, which may reflect practice effects since pretreatment deficits were not observed.

Discussion: These findings indicate minimal cognitive impairment among adult ambulatory outpatients with depression. Oculomotor

measures of voluntary motor control and sensorimotor function were sensitive to effects of depression and treatment recovery, while neuropsychological measures were not. The SSRI sertraline improved the ability to quickly plan and initiate behavior to a greater extent than other treatments, suggesting a possible beneficial effect of enhancement of serotonergic transmission on frontostriatal systems supporting the speed and precision of voluntary behavior.

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81. Maternal Brain Responses to Baby-Stimuli are Modulated by Psychopathological Risk

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Background: Parenting constitutes an evolutionarily conserved set of attachment behaviors and associated thoughts that contribute to responses to a distressed infant. These include immediate attention to needs and safety as well as soothing and caring that shape child development. Parenting behaviors and thoughts are influenced by a mother's own life experiences and repertoire of parenting-related thoughts. Upon hearing the distress signal of baby-cry, maternal neurophysiological systems respond to ensure adaptive responses, including increases in motivation to approach and decreases avoidance drives as a function of parenting-related thoughts. However, the capacity of mothers to generate caring behaviors and thoughts may be compromised by the accumulation of risk factors, including a history of child abuse, post-traumatic stress disorder, and/or major depression disorder. Thus, in this functional magnetic resonance imaging (fMRI) study, we hypothesize that: 1. Mothers brain responses to baby-cry, as a function of psychopathological risk, will be associated with decreased in positive motivation, rewarding and caring regions and increased avoidance and fear regions 2. Mothers at risk for depression will have inhibited self-reflection/empathy brain responses in response to personalized messages about parenting.

Methods: Eighteen mothers of 2-7 year old children were assessed for current and cumulative psychopathological risk, including histories of child abuse, post-traumatic stress disorder, major depression. In addition, the working model of the child interview (WMCI) was administered. In a Phillips 3T scanner, the participants underwent two types of task in a pseudo-randomized block-design: 1. An auditory baby-cry task, in which mothers listened to 30 second-blocks of 3 conditions and a pattern-matched white noise preceded by one of three primers: "a baby crying", "your baby crying", or "you yourself are crying as a baby". 2. A personally tailored message task, in which mothers were shown excerpts from their own responses to the WMCI, administered within a few days of the brain scanning over two conditions: directed feedback (e.g., "You find it most difficult to handle when [child's name] screams.") and open-ended feedback (e.g., "Think about when [child's name] screams."). In addition there was a non-parenting-related control message condition (e.g., "Think about the speed of the internet"). All data were analyzed with SPM 8.

Results: For the baby-cry task, we found the following: Listening to "a baby-crying" vs. white noise differentially activated salience-related regions of extended amygdala and insula, and that these neural responses increased with cumulative psychopathological risk. Listening to "your baby crying" vs. "a baby crying" differentially activated reward-related and salience regions of nucleus accumbens, and hippocampus. Listening to "you yourself as a baby crying" vs. "a baby crying" differentially activated anxiety- or stress-related regions of middle frontal gyrus, caudate, posterior insula, and habenula proportionally with cumulative psychopathological risk. To benchmark the neural regulation between positive and negative motivations, we contrasted "you yourself as a baby crying" with "your baby crying" and, as predicted, found that mothers with higher cumulative psychopathological risks showed reduced neural responses in the nucleus accumbens and hippocampus associated with positive motivation, but enhanced neural responses in the hypothalamus, midbrain, amygdala, caudate, anterior cingulate cortex (ACC), insula, and habenula associated with negative emotions of fear and avoidance: For the maternal interview task, we contrasted directed + open-ended feedback vs. control to find differential activation in the self-reflection regions of the dorsomedial prefrontal cortex, precuneus, posterior cingulate (PCC) cortex, and ACC. However, grouping subjects according psychopathological risk showed reductions in neural response in PCC and precuneus in to high vs. low risk. These results suggest a link between maternal psychopathology and diminished neural response in brain regions that are integral to self-reflection.

Discussion: Human parenting involves behaviors driven by key stimuli like baby-cries, as well as thoughts about being a parent. This work makes use of novel neuroimaging tasks to explore the brain activity underlying behaviors and thoughts across psychopathology risk. Previous mood and anxiety appear to alter brain responses important for parenting, suggesting opportunities for intervention and improved child mental health.

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82. The Behavioral and Functional Anatomical Correlates of Autobiographical Memory Deficits Associated with Depression Extend to Individuals at High Risk for Developing Depression

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Background: Patients with major depressive disorder (MDD), compared to healthy controls (HCs), consistently recall fewer *specific* autobiographical memories (AMs), defined as episodic memories of personally experienced events occurring in a period of no longer than one day, and instead recall more *categorical* AMs, defined as summaries of recurring events. This abnormality persists despite remission of depressive symptoms suggesting that this effect is has a biological rather than learned basis. In healthy humans AMs are known to activate a specific neural network that includes the hippocampus, precuneus, anterior cingulate cortex (ACC), and dorsolateral and ventrolateral prefrontal cortex (VLPFC). This study has two goals: 1) to examine the gross

heritability of AM overgenerality by determining if healthy subjects at a high-risk (HR; first-degree family relative with MDD) for developing MDD also show an overgenerality bias, and 2) to assess the differences in the functional neuroanatomical correlates of AM recall between MDD subjects, subjects at a high familial risk for developing MDD, and HCs.

Methods: The depressed MDD subjects (N = 16), HCs (N = 34), and HRs (N = 17) underwent fMRI while recalling AMs in response to 20 positive, 20 negative, and 20 neutral cue words. Subjects were presented with a cue word for 12 s and instructed to recall a past experience. Subjects then indicated the type of memory recalled (specific, categorical, extended, semantic, none) and the memory's valence.

The AM recall condition was compared to a semantic recall condition to control for abstract/general knowledge retrieval. In this control task subjects had 12 s to think of seven examples of a given category (e.g., tools). Ten positive, 10 negative, and 10 neutral categories were presented. They then rated the ease with which they generated the examples on a six-point scale and indicated the number of examples generated. Subjects had 10 s to make each memory/category rating. Following each cue/category word and each set of ratings the subjects engaged in a riser detection task to control for visual input/attention. All cue/category words were scrambled into nonword letter strings, and subjects were instructed to count the number of risers within the string. The presentation of each string was jittered with an average presentation time of 6 s. For half of these strings subjects had 2 s to indicate whether the number of risers was odd or even. Structural and functional imaging was conducted on a 3T GE Discovery MR750 MRI scanner with an 8-channel receive-only brain array coil. Gradient-recalled echoplaner imaging with sensitivity encoding (SENSE) was used for fMRI with the following parameters: 40 axial slices, TR/TE = 2000/25 ms, SENSE acceleration = 2, flip angle = 90°, matrix = 96 × 96, FOV/slices thickness/gap = 240/3/0 mm, number of volumes per run = 211). fMRI data were processed using AFNI. Group comparisons were performed with 3dANOVA and the significance threshold set at ($p_{uncorrected} < 0.001$).

Results: Behavioral results showed fewer specific and more categorical AMs in both the MDD and the HR samples compared to the HC sample. When comparing the hemodynamic changes during specific AM recall to those associated with semantic example recall, significant group differences were evident in prefrontal and temporal regions. HCs had greater activity in the hippocampus than either MDD or HR subjects, while HR subjects had more activity in the OFC and posterior cingulate cortex compared to MDD and HC subjects. Finally, the MDD subjects showed greater BOLD activity than the HR and HC groups in the VLPFC, ACC, precuneus and superior and inferior temporal gyrus versus HC and HR subjects.

Discussion: We replicated previous findings of fewer specific and more categorical AMs in depressed MDD subjects versus HCs, and for the first time demonstrate that these deficits exist in individuals at high familial risk for MDD. These data thus suggest that AM impairments constitute trait-like abnormalities in MDD. We also found distinct patterns of activation for each participant group as they recalled specific AMs. Importantly, the hippocampus was more active in HCs than in the other groups, indicating this core component of the AM network functions abnormally in both actively depressed patients and HR subjects. The HR subjects showed exaggerated BOLD responses in PFC areas relative to controls, possibly indicating these subjects expend greater effort to recall AMs. Similarly, MDD subjects showed greater BOLD activity than HCs in several core components of the AM network, possibly indicating that successful retrieval of specific memories in MDD patients requires greater effort or neural processing.

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83. Selective Serotonin Reuptake Inhibitors (SSRI) in Pregnancy: Current Knowledge of Effects on the Offspring

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Background: SSRIs are the most frequently prescribed drugs for depressive and anxiety disorders, in part because of their proven safety in adults. Even though none of the antidepressant drugs are approved for use in pregnancy, a study conducted in 2003 found that 13.4% of pregnant women were taking antidepressants. The safety of antidepressant use in pregnancy remains under debate. Pharmacological treatment in pregnancy requires weighing the risks and expected benefits for each individual patient. For this reason, it is important to provide access to up-to-date information on the safety of antidepressant drug use in pregnancy.

Methods: We present an overview describing potential adverse neonatal and postnatal consequences of gestational SSRI exposure based on prospective and retrospective cohort studies, on analyses of health databases and meta-analyses of the literature.

The following SSRI drugs, their dosages and duration of use will be reviewed: fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram.

Measures: 1) Birth outcome: physical development: gestational age, preterm births, birth weight and length. Neonatal adjustment: symptoms, Apgar scores, neonatal care unit (NICU) admissions, major and minor congenital malformations. 2) Postnatal development in infancy and childhood.

Results: Pharmacokinetic studies have shown that SSRI reach the fetus via the placenta. SSRI drug and metabolite concentrations have been detected in 68% of umbilical cord samples, with the umbilical cord serum/maternal serum ratio ranging from .29-.89. Maternal SSRI doses have been shown to correlate with umbilical cord and newborn serum drug and drug metabolite concentrations. Birth outcome: Gestational exposure to SSRIs may increase the risk for miscarriages (RR1.3-1.9). Compared to unexposed controls, most studies find no increased risk for major congenital malformations, except 6/7 studies report an increase in congenital cardiac malformations following first trimester exposure to SSRIs. The risk for minor malformations may or may not be increased. Persistent pulmonary hypertension of the newborn has been associated with exposure to SSRIs, however mode of delivery (Cesarian section) may contribute to the findings. Gestational SSRI exposure increases the risk for a shorter gestational age, more preterm births and low birth weights. 16/18 studies reported signs of poor neonatal adjustment (i.e. jitteriness, tremor, rigidity, hypoglycemia), lower Apgar scores at 1 and 5 minutes or more frequent NICU admissions.

Postnatal development: Studies (10/10) in infancy and childhood have found no differences in mental, cognitive or language development, however 4/6 studies reported a small delay in motor development after prenatal exposure to SSRIs.

Discussion: The majority of newborns prenatally exposed to SSRIs are born healthy. However, gestational exposure to SSRI antidepressants can affect fetal growth and development. The studies suggest a small risk for congenital cardiac malformations and for low birth weight and preterm births. Signs of poor neonatal adaptation are not infrequent and even if the symptoms tend to be transient, they often require clinical care. Too few studies have explored the long-term neurobehavioral effects of prenatal SSRI exposure. Mental development in infancy and early childhood has been found to be within the normal range, but there is evidence that SSRI may have small effects on motor development. More long-term outcome studies beyond early childhood are required to understand the implications of SSRI use in pregnancy for infant development.

Disclosure: R. Casper: None.

84. Change in Glucose and Lipid Metabolism using Stable Isotope Tracing During Euglycemic Clamp Conditions during Initial Antipsychotic Treatment for Disruptive Behavior in Youth

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Background: Rates of prescription of antipsychotic medications in children have increased, largely driven by use for disruptive behavior disorders. The effect of antipsychotic treatment on metabolic risk in antipsychotic naïve children has received little study, with investigations limited to case reports, non-randomized trials or randomized trials not specifically designed to measure metabolic changes as a primary outcome and/or not employing sensitive, gold standard metabolic measures. The NIMH-funded MEAC study (PI Newcomer, MH 072912) characterized the effects of 12 weeks of randomized antipsychotic treatment on direct measures of adiposity and insulin sensitivity (SI) in previously antipsychotic-naive children, permitting assessment of treatment-related changes in adiposity and associated changes in SI. We previously reported significant adverse changes in adiposity and insulin sensitivity during 12 weeks of treatment. Gold standard techniques, such as whole body dual energy x-ray absorptiometry (DEXA) to quantify adiposity combined with stable isotope tracer methodology during hyperinsulinemic-euglycemic clamp conditions can be used to evaluate whether observed changes in fat mass are functionally significant, for example leading to tissue-specific changes in SI.
Methods: Antipsychotic-naive participants aged 6-18 with clinically significant aggression and irritability (score of $\geq$18 on Aberrant Child Behavior Checklist Irritability Subscale) and one or more DSM IV diagnosis indicating a disruptive behavior disorder were enrolled and randomized to 12 weeks of treatment with aripiprazole, olanzapine or risperidone following baseline assessments. Baseline and 12 week measures included body composition analysis with Dual Energy X-ray Absorptiometry (DEXA), as well as metabolic testing including a single stage hyperinsulinemic-euglycemic glucose clamp using stable isotopomer tracing. SI at adipose tissue was measured by evaluating the rate of appearance (Ra) of labeled glycerol; SI at liver was measured by evaluating rate of appearance (Ra) of labeled glucose; SI at muscle was measured by evaluating the rate of disappearance (Rd) of labeled glucose. Regression analyses were performed to test the predictive effect of baseline DEXA-measured total % body fat on baseline insulin-stimulated reduction in glycerol Ra and glucose Ra and insulin-stimulated increase in glucose Rd; regressions were also performed to test the predictive effect of change in DEXA-measured total % body fat over 12 weeks on the change in insulin-stimulated reduction in glycerol Ra and glucose Ra, and in insulin-stimulated increase in glucose Rd.
Results: In a sample of 97 MEAC participants, pooling treatment groups to test the relationship of baseline and change in adiposity to baseline and change in insulin sensitivity, respectively, the magnitude antipsychotic treatment-induced increases in adiposity over 12 weeks of treatment were associated with the magnitude of adverse changes in SI at both adipose and hepatic tissues. Specifically, change in DEXA-measured total % body fat significantly predicted the capacity of insulin to decrease free fatty acid release, as measured by change in glycerol Ra ($F[1,95] = 4.973$, $p = 0.028$). A trend level relationship was observed between the magnitude of increase in total % fat over 12 weeks and the capacity of insulin to decrease hepatic glucose release, measured as glucose Ra ($F[1,95] = 2.839$, $p = 0.095$). Baseline relationships between adiposity and tissue-specific SI were also observed.
Discussion: Results from the MEAC study indicate rapidly detectable adverse effects of antipsychotic treatment on body composition (increased adiposity) and gold standard measures of glucose and lipid metabolism. Observed changes in DEXA total % fat are functionally significant, predicting reductions in the capacity of insulin to regulate glucose and lipid metabolism. The results suggest a key mechanism by which antipsychotic treatment can increase cardiometabolic risk during extended treatment. The results underline the importance of careful attention to the balance of potential risks and benefits during use of antipsychotic treatment in pediatric populations.
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Disclosure: J. Newcomer: Part 1: AstraZeneca Pharmaceuticals; Bristol-Myers Squibb; BioVail; H. Lundbeck; Janssen Pharmaceutical; Obecure; Otsuka Pharmaceuticals; Pfizer, Inc.; Sepracor, Inc.; Solvay Pharma, Inc.; Teva Pharmacutical; Vanda Pharmaceutica; Wyeth Pharmaceuticals; Dainippon Sumitomo Pharma America, Inc.; Organon Pharmaceuticals USA Inc.; Schering-Plough/Merck; Vivus, Inc.; Compact Clinicals/Jones and Bartlett Publishing Drinker, Biddle & Reath; Part 2: Vivus, Inc.; Dainippon Sumitomo Pharma America, Inc.; Organon Pharmaceuticals USA Inc.; Drinker, Biddle& Reath, Part 4: The National Institute of Mental Health (NIMH); NARSAD; Janssen; Bristol-Myers Squibb; Pfizer, Inc.l; Wyeth. **G. Nicol:** None. **M. Yingling:** None. **J. Schweiger:** None. **K. Flavin:** None. **M. Hessler:** None.

85. Relationship of Change in Adiposity to Psychiatric Symptom Change during Randomized Initial Antipsychotic Treatment in Pediatric Disruptive Behavior Disorders

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Background: Antipsychotic treatment commonly increases adiposity in children and adults, leading to associated changes in insulin sensitivity and lipid metabolism. A positive predictive relationship between weight gain and therapeutic efficacy during antipsychotic treatment has been reported in schizophrenic adults (1), despite well-known adverse health effects associated with weight gain in this population (2). The relationship between weight gain and treatment response in youth, now commonly treated with antipsychotic agents for disruptive and/or aggressive behaviors, has not been evaluated. The relationship between early onset of obesity and cardiometabolic risk factors like impaired glucose tolerance and hyperlipidemia, known to predict cardiovascular morbidity and mortality in adulthood, support the need to study the relationship between changes in body composition and treatment response in youth treated with antipsychotics.
Methods: Hypotheses regarding changes in body composition predicting therapeutic response to antipsychotic were tested using data from the MEAC study (Metabolic Effects of Antipsychotics in Children, MH72912, PI Newcomer). Antipsychotic naïve children ages 6-18 ($N = 144$) presenting with disruptive or aggressive behavior in the setting of one or more eligible DSM-IV diagnoses were randomized to 12 weeks of treatment with olanzapine, risperidone, or aripiprazole, with pooled treatment group changes analyzed. Changes in body composition were evaluated using baseline and at endpoint Dual Energy X-Ray Absorptiometry (DEXA), abdominal Magnetic Resonance Imaging (MRI), Body Mass Index (BMI), BMI percentile, BMI z-score and waist circumference (WC).

Changes in psychiatric symptoms were measured using the Aberrant Behavior Checklist (ABC) irritability, hyperactivity and total score.
Results: Change in both ABC irritability subscale and ABC total scores significantly predicted changes in BMI percentile ($F[1,126] = 10.448$, $p = 0.002$; $F[1,126] = 11.509$, $p = 0.001$), BMI z-score ($F[1,126] = 8.203$, $p = 0.005$; $F[1,126] = 9.218$, $p = 0.003$), DEXA % body fat ($F[1,126] = 4.276$, $p = 0.041$; $F[1,126] = 4.765$, $p = 0.31$) and DEXA % lean mass ($F[1,126] = 4.353$, $p = 0.039$; $F[1,126] = 4.804$, $p = 0.030$). BMI%ile ($F[1,126] = 6.586$, $p = 0.011$) and BMI z-score ($F[1,126] = 4.998$, $p = 0.027$) also significantly predicted change in ABC hyperactivity scores. However, the amount of variance explained for all significant effects was very small and the directionality of all significant effects except those for DEXA % lean indicated a tendency for symptoms to worsen with increasing mass. Only a minority of participants increased % lean mass, and only by a small amount (all $<5\%$), limiting the clinical significance of this result. No significant relationship was observed between change in body weight, WC or abdominal fat and change in ABC irritability, hyperactivity or total scores.
Discussion: These results are relevant to the evaluation of potential risks and benefits during treatment with antipsychotic medications in youth treated for disruptive behaviors, one of the most common reasons for pediatric antipsychotic prescriptions in both privately and publicly insured populations. The results indicate that commonly occurring increases in adiposity during initial antipsychotic treatment are not associated with clinical improvement, with a tendency for poorer treatment outcomes in relation to greater increases in adiposity.
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Supported by MH72912, this research was also made possible by Grant Number P30DK056341 from the National Institute of Diabetes And Digestive And Kidney Diseases and Grant Number UL1RR024992 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research. Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIDDK, NCRR or NIH.
Disclosure: G. Nicol: Part 4: Pfizer Inc., Bristol Myers Squibb, Sidney R. Baer, Jr. Foundation. **M. Yingling:** None. **K. Flavin:** None. **J. Schweiger:** None. **M. Hessler:** None. **J. Newcomer:** None.

86. Pharmacogenetics of Glutamate System Genes and SSRI-Associated Sexual Dysfunction

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Background: Sexual dysfunction is a common side effect experienced by patients using selective serotonin reuptake inhibitors (SSRIs). Single nucleotide polymorphisms (SNPs) in *GRIA3* (glutamate receptor, ionotropic, AMPA3), *GRIK2* (glutamate receptor, ionotropic, kainate2), and *GRIA1* (glutamate receptor, ionotropic, AMPA1), were associated with decreased libido and difficulty with orgasm in individuals treated with the SSRI citalopram in a previous study. We further studied the relationship between these SNPs and SSRI-associated sexual dysfunction using a validated measure for assessing sexual dysfunction in a patient population devoid of other medications and medical comorbidities that may influence sexual function outcomes.
Methods: One hundred and fourteen subjects with no history of sexual dysfunction prior to medication initiation (76% female), aged 26.2 ± 5.7 years, who had received an SSRI at least 6 weeks for depression, were evaluated in this point prevalence pharmacogenetic study. Relationships between clinical, medication, and Changes in Sexual Functioning Questionnaire (CSFQ) variables were assessed. Associations between polymorphisms in *GRIK2* (rs9404130, rs513216), *GRIA3* (rs2285127, rs2269551, rs550640), and *GRIA1* (rs1994862, rs10515697, rs1864205) and sex-specific thresholds for dysfunction as measured by CSFQ total and subscale measures were examined.
Results: Sexual dysfunction measured by falling below sex-specific CSFQ total score thresholds (36%) did not differ across medications but was more common in females (42%) than males (17%) ($p < 0.05$). Depressive symptoms as measured by the Hamilton Depression Rating Scale-21 scores (5.9 +/-3.2) were minimal and not differ across glutamate genotype groups. The *GRIA1* rs1994862_AA SNP was significantly associated with a lower risk for arousal dysfunction (OR = 0.16, 95% CI 0.03, 0.8) and remained significant after controlling for multiple comparisons ($p < 0.05$). There was no evidence for significant genotype by sex interactions ($p > 0.05$ for all interaction terms). While not reaching statistical significance, unadjusted results for rs550640 ($p = 0.07$ on the arousal subscale), rs513216 ($p = 0.08$ on total score, $p = 0.13$ on the orgasm subscale), and rs9404130 ($p = 0.10$ on the orgasm subscale) were suggestive of relationships for further study.
Discussion: Our results support the association of a common SNP in the *GRIA1* gene (rs1994862) with sexual dysfunction in patients taking an SSRI for depression. This SNP was found to be significantly associated with sexual desire in a previous study that also identified associations between the other glutamate system SNPs and sexual dysfunction. Unadjusted findings for other SNPs (rs550640, rs513216, and rs9404130) indicate that they may also be involved with SSRI-associated sexual dysfunction and warrant further analysis in future studies.
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87. Deep Brain Stimulation Research for Treatment-Resistant Depression: Empirical Investigation of Ethical Concerns

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Background: Deep brain stimulation (DBS) is being actively investigated as a potential treatment for patients with treatment-resistant depression (TRD). Concerns have been expressed about the research-related decision-making abilities of patients with TRD, including worries that participants' decision making might be unreasonably motivated by expectations of personal benefit, minimization of risks, or desperation. Several prior studies have examined decision-making abilities of patients with depression, but none of these focused on patients with TRD or on depressed patients considering enrollment in research involving a neurosurgical intervention.
Methods: This study examined the decision-making abilities, influences on decision making for research participation, and perceptions of risks and benefits of 28 adults who were potential participants in two separate studies of DBS for TRD (a foundation-funded, investigator-initiated study and an industry-sponsored clinical trial). Decision-making abilities were assessed as part of the informed consent procedure, using the MacArthur Competence Assessment Tool for Clinical Research (MacCAT-CR), a semi-structured interview designed to evaluate decisional capacity. The MacCAT-CR has four subscales assessing four components of decision-making capacity: Understanding, Appreciation,

Reasoning, and Expression of a Choice. Scores on each subscale were calculated, along with correlations of MacCAT-CR subscale scores with depression scores. Qualitative data were also collected in the form of open-ended responses to specific prompts regarding perceived risks and benefits of participation. Finally, thematic analyses were conducted regarding influences on decision making, based on responses to questions posed in the MacCAT-CR.
Results: The mean score on the Understanding subscale was 35.7 (SD = 2.5) out of a maximum of 38, with no subject scoring below 27. The mean score on the Appreciation subscale was 5.9 (SD = .4) out of a maximum of 6, with no subject scoring below 4. The mean score on the Reasoning subscale was 7.8 (SD = .6) out of a maximum of 8, with no subject scoring below 6. There was no correlation between performance on any of the three MacCAT-CR subscales and degree of depressive symptoms or demographic characteristics. Themes identified based on qualitative data suggested possible misunderstandings, particularly with regard to individual perceptions of risk and expectations of benefit. Participants cited numerous factors as influential in their enrollment decisions, including perceived lack of other treatment options, desire to take initiative, beliefs about DBS as a novel treatment, possibility of DBS efficacy, hoped-for improvements, potential risks and disadvantages of DBS or clinical trial participation, and altruism. No participant expressed motivations or described factors influencing the decision that suggested frankly compromised decision-making capacity or diminished voluntariness.
Discussion: Participants in these early-phase DBS trials for TRD showed few impairments in their decision-making capacities related to enrollment. These findings are consistent with prior research on capacity to consent to research among patients with depression and other mental illnesses. These results suggest that potential subjects make the decision to enroll in early-phase trials of DBS for TRD based on a number of complex and sometimes idiosyncratic considerations, and that the trials that were studied utilized sufficiently robust informed consent processes. These findings offer evidence that the emerging research area of DBS can be advanced in an ethically sound manner, provided that safeguards and processes for discussing trials with participants are carefully developed and proactively put into place. Although the applicability of these findings to research on other innovative treatments for TRD (and perhaps other psychiatric disorders) is unknown, they offer some reason for optimism that effective informed consent may be possible in those contexts as well. Concerns about decision-making capacity of people with psychiatric disorders must be subjected to empirical assessment rather than being accepted at face value. Empirical ethics research therefore is an integral part of the research endeavor, helping to reduce stigma of research and better characterize the strengths of patients with psychiatric disorders while providing important information about ways in which safeguards are working or can be improved.
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88. Neuron-Specific Deletion of Histone Methyltransferase Mll1 is Associated with Behavioral Deficits and Altered Histone Methylation at Neuronal Gene Promoters

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Background: Mll1 (mixed-lineage leukemia) methyltransferase specifically regulates trimethylated histone H3 on lysine K4 (H3K4me3) associated with active gene expression. Because the cerebral cortex of some patients with schizophrenia shows altered histone methylation signatures and decreased expression of GABAergic genes, we tested whether neuron-specific deletion of Mll1 in mice would lead to behavioral changes indicative of cortical dysfunction.
Methods: Conditional Mll1 mutant mice were generated using the loxP Cre-recombinase system under the control of the CaM kinase promoter, leading to Mll1 deficiency in forebrain neurons. Adult mutant and control mice were subjected to behavioral tests relevant for function of the frontotemporal-hippocampal network: working memory, anxiety, locomotor activity, nest building and psychomotor activities. Genome-wide next generation sequencing was used to map histone methylation landscapes of cortical neuronal nuclei from Mll1 deficient mice and littermate controls.
Results: Conditional Mll1 mutant mice showed reduced working memory, enhanced locomotor activity and anxiety, and impaired nest-building behavior. Approximately 200 loci in the genome were affected by H3K4me3 alterations in the Mll1 deficient neurons. Intriguingly, one quarter of these loci showed strong drifts in H3K4me3 levels during the course of normal postnatal development.
Discussion: Mll1 deficient mice have a phenotype indicative of impaired cortico-limbic networks, which could be, at least in part, explained by dysregulated epigenetic remodeling of neuronal genes during postnatal development of cerebral cortex. Taken together with related findings from human prefrontal cortex, these studies further emphasize that fine-tuning of histone methylation is important for an extended period of brain maturation and could play a role in the pathophysiology of neurodevelopmental disease.
Disclosure: M. Jakovcevski: None. **W. Mao:** None. **I. Cheung:** None. **C. Connor:** None. **J. Straubhaar:** None. **S. Akbarian:** None.

89. Emotional Modulation of Response Inhibition in Unaffected Siblings of Patients with Bipolar I Disorder

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Background: Investigations of endophenotypes in bipolar disorder (BPD) have identified a number of neurocognitive deficits as potential vulnerability markers of the disorder. Attention and executive deficits have been exhibited by both euthymic BPD patients and unaffected relatives of BPD probands in processing speed, verbal learning, set-shifting, and executive control. One neurocognitive domain that has been the subject of a substantial literature in BPD, but not widely explored as a

potential endophenotype of the disorder, is affective, or emotional, processing. Adult and pediatric BPD patients have exhibited impairments and biases in facial affect recognition and a recent study suggests that young individuals at risk for BPD show a generalized insensitivity to facial affect.

We recently reported that stable BPD patients demonstrate a trait-like response bias toward negative affective stimuli on an affective go/no-go task as compared with healthy controls and schizophrenia patients (Gopin *et al.*, 2011). The goal of the current study was to expand upon these prior findings to investigate these patterns in the unaffected siblings of BPD patients to determine the extent to which the affective bias may be associated with genetic predisposition for the illness.

Methods: 20 BPD I patients, 20 of their unaffected siblings, and 20 healthy controls (HC) were included. Unaffected siblings had no evidence of Axis I disorder (as per SCID), were past the modal age of onset for BPD ($>$ 25 years), and were not more than two years younger than their affected sibling was at the time of his/her illness onset. These criteria were set in place to reduce the likelihood that the unaffected sibling group would include individuals who would go on to later develop the disorder. The HC subjects ($n = 20$) were free from Axis I pathology and had no first degree relatives with an Axis I disorder. All participants received clinical ratings for mania and depression at the time of assessment.

An affective Go/No-Go test was used to evaluate inhibitory response to negatively-valenced (gun, rain), positively-valenced (sunshine, money), and neutral stimuli (house, store). Accuracy (d′) and response bias (beta) served as dependent variables in a series of repeated measures ANCOVAs with group as a fixed factor.

Results: Subject groups were demographically well-matched but differed in estimated premorbid IQ; therefore, this variable served as a covariate in all subsequent analyses. Unaffected siblings showed a response bias (beta) toward negatively valenced stimuli vs. healthy controls [$F = 3.97$; $p = .028$], a very similar pattern to that seen in a larger cohort of stable BPD patients from our prior work. Likewise, as noted in the BPD subjects, we found no significant group effects on accuracy performance (d′) for any of the conditions (negative, positive or neutral) when comparing unaffected siblings vs. healthy controls [$F = .984$; $p = .384$].

Discussion: While other cognitive skills such as sustained attention and verbal memory have been identified as endophenotypes of BPD, no study to date has implicated biased affective processing as a potential biomarker. Our results revealed a significant response bias towards negative stimuli in the unaffected siblings of BPD patients when compared with unrelated healthy controls. In light of our previous work showing a similar effect for stable BPD patients, this finding implicates affective processing bias as a potential endophenotype in BPD, and supports future efforts to replicate and expand upon these findings.

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90. Whole Exome Sequencing in First Degree Cousin Pairs with Early Age-at-Onset Bipolar Disorder

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Background: Though bipolar disorder is highly heritable, recent estimates of total genetic variance accounted for by common variants have been low. Identification of uncommon and rare variants may increase the total genetic variance explained.

Methods: In this pilot study, we identified 4 first-cousin pairs with bipolar I disorder beginning before age 16. Cases were drawn from 4 distinct families of European-ancestry ascertained by the NIMH Genetics Initiative. Exome sequencing was performed with SOLiD technology. Fragment libraries were made with 5 ug genomic DNA, followed by exome capture using the Agilent SureSelect Human All Exon 50MB kit. Between 95 M and 120 M single-end reads were generated per individual (4 exomes/SOLiD Quad). Of these, 65 M-80 M reads mapped to the reference genome (hg19). Duplicated reads were removed using PICARD, and non-duplicated reads were re-calibrated using GATK. Of 82K called SNVs, $\sim$26K were filtered out due to quality control or minor allele frequencies $>$10% in the 1000 Genomes Database. The remaining $\sim$56K variants were then mapped to regions of the genome shared identical-by-descent (IBD) between cousin pairs, using BEAGLE IBD.

Results: The proportion of single nucleotide variants shared by cousin-pairs exceeded the proportion of the genome shared IBD by 150 to 176%. Approximately 147 variants mapped within IBD regions in all 4 cousin pairs, 100% of which could be phased. One variant, predicted to be damaging by SIFT, was found to reside on the haplotype that was actually shared IBD within that cousin pair.

Discussion: High throughput sequencing of cousins with early onset bipolar disorder reveals an excess of single nucleotide variants within shared genomic regions. Uncommon and rare variants may provide new insight into the etiology of bipolar disorder.

Disclosure: D. Chen: None. **N. Akula:** None. **L. Kassem:** None. **F. McMahon:** None.

91. Association of Genetic Variants with Baseline Pain in Patients with Major Depressive Disorder

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Background: Previously, Diatchenko and colleagues described associations between a haplotype marker in the gene coding catechol-O-methyltransferase (*COMT*; haplotype marker consisting of variants in rs6269, rs4633, rs4818, and rs4680) (1) as well as a haplotype marker in the gene coding adrenergic receptor beta 2 (*ADRB2*; haplotype marker consisting of variants in rs11958940, rs1432622, rs1432623, rs2400707, rs1042713, rs1042714, and rs1042717) (2) with pain sensitivity in female carriers. Here, we tested the hypothesis that those previously described haplotype markers in *COMT* and *ADRB2*, as well as single-nucleotide polymorphisms (SNPs) in *COMT* and 5-hydroxytryptamine receptor 3A (*HTR3A*) which were associated with depression response during treatment with duloxetine in prior studies, are associated with baseline pain in patients with major depressive disorder (MDD).

Methods: We analyzed data from self-identified white patients ($N = 258$) of either gender who agreed to genetic testing while participating in a 12-week randomized clinical trial that compared open-label treatment with duloxetine and selective serotonin reuptake inhibitors (SSRIs) in patients with MDD. We tested associations between baseline mean Brief Pain Inventory (BPI) average 24-hour pain scores and *COMT* and *ADRB2* haplotype markers as well as *COMT* (rs174696) and *HTR3A* (rs1176752 and rs1150226) SNPs. To account for potential gender differences, data from male and female patients were analyzed combined and stratified by gender.

Results: All patients were diagnosed with MDD according to criteria described in the *Diagnostic and Statistical Manual of*

Mental Disorders – Fourth Edition – Text Revised (DSMIV-TR). The cohort used in the current analyses was mainly female (68.6%) with a mean age of 45 years and a baseline mean BPI average 24-hour pain score of 3.66. Baseline mean BPI average 24-hour pain scores did not demonstrate any statistically significant associations with previously described haplotype markers in *COMT* and *ADRB2* or with SNPs in *COMT* and *HTR3A* in the combined patient population or after stratification by gender. However, male patients with 1 haplotype coding for low and 1 haplotype coding for high *ADRB2* expression (H1H2 or H1H3) ($n = 33$), previously reported to be associated with less vulnerability to developing chronic pain in female carriers, had higher mean baseline mean BPI 24-hour average pain scores (mean baseline BPI 24-hour average pain score = 4.4) than male carriers of all other *ADRB2* genotypes (mean baseline BPI 24-hour average pain score = 2.9, $n = 41$, $p = 0.019$). The percentage of male patients with mean baseline BPI 24-hour average pain scores of 6 or more was nearly 3-times higher in male H1H2 or H1H3 carriers compared with male carriers of all other *ADRB2* genotypes, while the percentage of male carriers of all other *ADRB2* genotypes with no baseline pain was nearly 3-times higher compared with male H1H2 or H1H3 carriers.

Discussion: In the current analyses, we were not able to replicate previously described associations between genetic polymorphisms and pain susceptibility. While a possible explanation might be type II error due to a relatively modest sample size and differences in study design, some prior findings might also be due to type I error considering the well-established high risk for false positives in genetic association studies. The observed association between baseline BPI 24-hour pain scores and the *ADRD2* haplotype marker in male patients is intriguing and warrants further examination in larger patient populations. Despite the difference in direction, this finding does not contradict the previous report by Diatchenko and colleagues who only examined female carriers. While we did not replicate the results of Diatchenko and colleagues in our female cohort, the results presented here provide preliminary information for a possible genetic basis to differentiate depressed male patients who are likely to have painful physical symptoms.

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92. Whole Genome Sequencing Identifies a Coding Sequence Variant in the NTRK1 Gene Segregating with Both Bipolar Disorder and Medullary Cystic Kidney Disease in an Unusual Family

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Background: We have previously reported identification of a family in which bipolar disorder co-segregated with autosomal dominant medullary cystic kidney disease (MCKD). MCKD is characterized by multiple renal cysts and has onset in the 20's with renal failure followed by dialysis or transplant usually by age 30. All six children of a father with both disorders inherited both MCKD and a mood disorder phenotype. This family was identified as part of a linkage study of bipolar disorder and a genome scan suggested linkage to several chromosomal regions including 1q, 12p, 13q, 19q and 22q. Regions of homozygosity in the father further suggested either inbreeding or partial uniparental disomy that explained these linkage signals.

Methods: In order to identify possible sequence variants responsible for both disorders, we sequenced the entire genome of the proband at 11x coverage using next generation sequencing technology.

Results: A coding sequence variant resulting in a glutamic acid to lysine substitution was identified and validated that segregated with both disorders in the family. This variant was located in the juxtamembrane region of the receptor and resulted in a change in charge. NTRK1 codes for TrkA, which is a receptor for nerve growth factor (NGF).

Discussion: A variety of mutations in the NTRK1 gene have been associated with inability to feel pain and anhidrosis, as well as, renal failure, but not previously to mood disorders. We and others have previously reported data implicating BDNF and its receptor TrkB in bipolar disorder and lithium response. These preliminary data suggest that TrkA may also convey vulnerability to bipolar disorder and add further evidence implicating the neurotrophin system.

Disclosure: J. Kelsoe: None. **T. Shekhtman:** None. **S. Szelinger:** None. **D. Craig:** None.

93. Differential Effects of BDNF and 5-HTT Polymorphisms on Interferon-Related Depression

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Background: Major depressive disorder is likely the result of the cumulative and interactive effects of several entities including genetic vulnerability. Increasing evidence demonstrates a role for inflammatory cytokines in the etiology of many instances of major depression. Consistently, many patients receiving interferon-alpha therapy develop depression within weeks or months of treatment. However, only about $\frac{1}{4}$ are vulnerable. We and others have found that 5-HTTLPR is associated with this vulnerability. Many cytokines can also influence brain-derived neurotrophic factor (BDNF) release, and some studies support a role for polymorphisms in BDNF in depression vulnerability. Therefore we examined

the influences of a functional Met/Val BDNF polymorphism and compared it to the effect of the 5-HTTLPR polymorphism.
Methods: A cohort of euthymic adult patients (n = 64) with hepatitis C, not taking any psychotropic medications, who were beginning interferon-alpha treatment were prospectively followed using the SCID-IV and Beck Depression Inventory (BDI). Serum samples at baseline and monthly were used to examine BDNF levels (ELISA), as well as genotype for 5-HTTLPR (S allele sv. L/L homozygotes) and the Val/Met BDNF genotype (Met allele vs. Val/Val homozygotes). Kaplan-Meier analyses compared incidence of depression between genotypes, and mixed-effect repeated-measure analyses were used to examine individual symptoms on the BDI.
Results: Both the Met allele and the S/S 5-HTTLPR genotype were associated with increased depression incidence (Mantel-Cox log rank test, $p < 0.05$). Appetite complaints for those with the S allele averaged 0.35 +/- 0.05 at baseline, increasing to 0.72 +/- 0.07 at month one and 0.73 +/- 0.18 at month three; compared to the L/L genotype at the respective time points – 0.19 +/- 0.10, 0.77 +/- 0.11, 0.45 +/- 0.13 ($F = 5.6$; $p < 0.005$). Similarly, the S allele was associated with the neurovegetative symptoms of worse sleep, loss of appetite, poor concentration, low libido, low energy, and loss of pleasure (all $p < 0.05$); but 5-HTTLPR was not associated ($p > 0.1$) with other depressive symptoms. Conversely, the Met allele had a 'sadness' baseline score of 0.16 +/- 0.08 which increased to 0.5 +/- 0.19 by month one and then to 0.75 +/- 0.35 by month three. The Val/Val genotype had scores of 0.13 +/- 0.05, 0.14 +/- 0.06, and 0.19 +/- 0.11 at similar time points ($F = 8.3$, $p < 0.005$). The Met allele was likewise associated ($p < 0.05$) with guilt, pessimism, self-dislike, self-criticalness, loss of interest, feelings of being punished, and poor concentration. In contradistinction to 5-HTTLPR, the BDNF genotype was not associated ($p > 0.1$) with any neurovegetative symptoms.
Discussion: The BDNF allele was not associated with neurovegetative symptoms of depression during interferon-alpha treatment (e.g., sleep/appetite/energy/libido), but was associated with more psychological and cognitive symptoms. Conversely, 5-HTTLPR was more specifically associated with the neurovegetative symptoms. This supports the notion that both serotonin and neurotrophic growth factors are involved in vulnerability to depression but suggests that each influences vulnerability through distinct pathways. There are implications for both treatment and measurement. For example, SSRIs have not been effective in treating the neurovegetative symptoms of interferon-induced depression (which are more prominent in those with the S allele); and 5-HTTLPR is not associated with interferon-induced depression when using measures such as the Hospital Anxiety-Depression Scale (HADS), which is designed to minimize the role of neurovegetative symptoms. Moreover, these implications may apply to other types of depression in which inflammatory cytokines may play a role.
Disclosure: F. Lotrich: None. **R. Ferrell:** None. **D. Sorisio:** None. **S. Albusaysi:** None. **D. Brent:** None.

94. Identification of Mitochondrial Somatic Mutations in Subjects with Mood Disorders and Schizophreni

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Background: The human mitochondrial DNA genome (mtDNA), which is independent from the nuclear genome, has been shown to accumulate mutations in the brain during the lifespan that might lead to biochemical dysfunctions and observable phenotypic changes. Interestingly, mitochondrial diseases are often characterized by the presence of psychiatric symptoms, thus, suggestive of a link between mutations in the mtDNA and susceptibility to psychiatric disorders.
Methods: Mitochondrial DNA (mtDNA) somatic mutations were investigated in schizophrenia (SZ, N = 10), bipolar disorder (BD, N = 10), major depressive disorder (MDD, N = 10), methamphetamine positive (Meth, N = 6), and controls (N = 10). We compared a 4977 base pair mtDNA common deletion between SZ, BD, MDD, Meth, and controls, across eleven brain regions (dorsolateral prefrontal cortex, orbitofrontal cortex, anterior cingulate, caudate, putamen, nucleus accumbens, substantia nigra, amygdala, hippocampus, thalamus, and cerebellum) by a covariate analysis for age.
Results: We found the presence of the common deletion to be highly variable and significantly different across the 11 brain regions. However, there were no significant differences in the 4,977 bp in eleven brain regions by diagnosis, although a significant effect of age was seen.
Discussion: This study reinforces prior research that the brain shows an increased accumulation of mitochondrial somatic mutations with age. The lack of differences in different disorders will be investigated with more precise cellular and layer resolution in order to assess possible clonal expansion in these brain regions that show highest levels of the somatic mutation.
Disclosure: F. Mamdani: None. **P. Sequeira:** None. **B. Rollins:** None. **W. Bunney:** None. **F. Macciardi:** None. **M. Vawter:** None.

95. CRHR1 Genetic Variation Potentiates Stress-induced Anhedonia: A 128-channel Event-related Potential Study

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Background: Stress has emerged as a key risk factor for psychopathology, including depression, but the mechanisms underlying this risk remain largely unknown. Preclinical data suggest that stress can induce anhedonic-like behavior. Moreover, growing evidence indicates that genetic variation within the corticotropin releasing hormone type 1 receptor (CRHR1) might increase risk for depression and negatively affect approach-related behavior. The goal of this study was to investigate in a psychiatrically healthy sample whether CRHR1 variants are associated with impairments in reinforcement learning under stress.
Methods: We recorded 128-channel event-related potentials (ERPs) while 75 Caucasian females completed a probabilistic reward learning task during both stress (threat-of-shock) and no-stress conditions. Response bias, an individual's ability to modulate behavior as a function of reward, was the primary behavioral variable of interest. The feedback-related positivity (FRP) – a frontocentral positive-going deflection elicited by positive prediction errors and rewards and hypothesized to originate from dorsal anterior cingulate cortex (ACC) and striatal regions implicated in reinforcement learning – was used as a neural index of reward learning. Low Resolution Electromagnetic Tomography (LORETA) – a distributed ERP source localization technique – was used to estimate intracerebral current density underlying the FRP. Using a single nucleotide polymorphism (SNP) tagging approach, we focused on a SNP previously linked to depression (rs12938031; Thode *et al.*, in press).
Results: Relative to the no-stress condition, acute stress was associated with blunted response bias, a smaller and delayed FRP (indicative of disrupted reward learning), and reduced activation to rewards in dorsal anterior cingulate regions previously implicated in integrating reinforcement over time. Critically, rs12938031 interacted with stress to influence reward learning: both behaviorally and neurally, A homozygotes showed stress-induced reward learning abnormalities.

Discussion: These findings indicate thatacute, uncontrollable stressors reduce participants' ability to modulate behavior as a function of reward, and that such effects are modulated by *CRHR1* genotype. Homozygosity for the A allele at rs12938031 may increase risk for psychopathology via stress-induced reward learning deficits. Implications for depression research will be discussed. Thode K, Walss-Bass C, Hariri AR, Olvera R, Muñoz K, Qureshi N, Beuten J, Gelfond J, Maher B, Dahl R, Birmaher B, Ryan N, Williamson DE (in press) Functional evidence implicating the role of the corticotropin-releasing hormone receptor 1 in development of stress-related disorders. Am J Psychiatry.
Disclosure: D. Pizzagalli: Part 1: Consulting fees: ANT North America Inc. (Advanced Neuro Technology), AstraZeneca, Ono Pharma USA, Inc. Honoraria: AstraZeneca, Part 4: NIH/NIMH. **R. Bogdan:** None. **R. Perlis:** Part 1: Honoraria, speaker's or consulting fees: AstraZeneca, Bristol Myers-Squibb, Eli Lilly & Co., Glaxo SmithKline, Pfizer, and Proteus. Major stockholder: Concordant Rater Systems, LLC., Part 4: NIH/NIMH Stanley Center for Research in Psychiatric Disease. **D. Santesso :** None. **J. Fagerness :** None.

96. COMT Gene Influences on Novelty Seeking Across Species: A Preliminary Study

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Background: Novelty seeking is defined as a heritable proclivity to approach unfamiliar stimuli or situations, and in its exaggerated form reflects a decreased ability to self-regulate behavior – a primary function of the frontal cortex. The trait of high novelty seeking is a major predictor of impulsive, addictive behaviors as well as activities that are associated with personal risk. Increased novelty seeking underlies many cardinal symptoms of psychiatric conditions such as Bipolar Disorder (BD), Attention-Deficit Hyperactivity Disorder (ADHD), substance use disorders, and other pathologies. Unlike other psychiatric symptoms, novelty seeking can be quantified in both humans and animals using parallel methodologies. One such measure is an "open field" paradigm termed the Behavioral Pattern Monitor (BPM) that is applied in both humans and rodents. Thus the measurement of novelty seeking can be subjected to translational research, which is hoped to lead to the eventual development of much-needed new treatments for BD, ADHD, and other conditions. The biological basis for this central feature of multiple diseases is not well understood, but genes that influence catecholamine signaling in the frontal cortex, such as the gene for the catechol-O-methyltransferase (COMT) enzyme, have been implicated. This study examined whether COMT genotype influences novelty seeking in humans in the manic phase of BD, putatively a hyperdopaminergic state, as well as in mice with the humanized COMT gene that received a catecholamine indirect agonist, methylphenidate. We hypothesized that the Methionine (Met) allele, which results in decreased activity of the enzyme and thus increased availability of catecholamines in the frontal cortex, would be associated with increased novelty seeking in manic BD humans and mice administered methylphenidate.
Methods: Adults diagnosed with BD, current episode manic ($n = 25$) were genotyped for the COMT Val158Met polymorphism and tested within 96 hours of admission to an inpatient psychiatric hospital. They were administered the human BPM (hBPM), a human open field test where subjects are placed into an unfamiliar room containing novel objects and given no instructions. Subjects' activity and interactions with objects are recorded with a digital videocamera. Genetically engineered mice with the human COMT gene Valine (Val) or Met allele replacing the mouse COMT gene were treated with either methylphenidate 10 mg/kg or vehicle and tested in the mouse BPM.
Results: Manic BD patients homozygous for the Met allele of COMT showed increased entries into areas of the hBPM that contained objects and Val homozygotes showed the least activity in these areas. Dose of Met allele was significantly positively correlated with multiple object interactions ($r = 0.50$, $p = 0.01$), time spent in object-proximal sectors ($r = 0.52$, $p = 0.01$), and number of entries into object-proximal sectors ($r = 0.42$, $p = 0.04$). Preliminary data in the F1 generation of COMT Val158Met mice suggest that mice homozygous for the Met allele exhibited significantly greater responses to methylphenidate compared to Val/Val mice in overall activity (counts),distance and geometrical path ($p < 0.05$ Val/Val vs Met/Met, one-tailed t-test $N = 7$-13).
Discussion: Our findings in both humans and mice are consistent with suggestions that the Met allele, in combination with a putative hyper-catecholaminergic state, may confer liability toward excessive novelty-seeking behavior, possibly because of tonic increases in frontal catecholamine levels which theoretically result in difficulty modulating responses to novelty in the environment. The mouse data must be regarded as preliminary since this experiment was in a genetically mixed background (129 ES cell line carriers bred to C57BL6J wild-type mice). We are currently creating a pure C57 background line for the proposed studies to ensure background genes do not confound our results. These preliminary data support the cross-species approach undertaken here, suggesting this strategy can be used to identify neural substrates underlying gene-phenotype relationships involved in novelty seeking and in the future may provide new treatment targets for humans with psychiatric conditions where novelty seeking is an essential feature.
Disclosure: A. Minassian: None. **V. Risbrough:** None. **X. Zhou:** None. **W. Perry:** None. **J. Kelsoe:** None. **M. Geyer:** Part 1: Dr. Geyer has received consulting compensation from Acadia, Addex, Cerca, Medivation, Merck, and Omeros, Takeda, and Teva, and holds an equity interest in San Diego Instruments. Dr. Geyer also has research grant support from Intracellular Therapeutics, Johnson & Johnson, NIDA, NIMH, and the U.S. Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center., Part 2: San Diego Instruments and Omeros., Part 3: None exceeds 5%, Part 4: Intracellular Therapeutics.

97. More Severe Longitudinal Bipolar Illness Course Associated with Early Life Stress in Brain-Derived Neurotrophic Factor Met Allele Carriers but not Non-Met Allele Carriers

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Background: Emerging findings support interaction effects of brain-derived neurotrophic factor (BDNF) val66met genotype with environmental factors, such that individuals who carry the met allele may be more susceptible to the affective consequences of stress. Previous studies found that a gene-by-environment interaction between BDNF met carrier status and early life stress (ELS) predisposed individuals in non-clinical samples to symptoms of depression. However, there are limited data regarding the impact of this gene-by-environment interaction on severity of illness in individuals diagnosed with bipolar disorder. We conducted a study to examine whether BDNF met carrier status moderates the negative impact of ELS upon longitudinal bipolar illness severity.
Methods: Stanford University Bipolar Disorders Clinic patients were initially assessed with the Systematic Treatment Enhancement for Bipolar Disorders (STEP-BD) Affective Disorders Evaluation (ADE), and monitored longitudinally for at least

one year with the STEP-BD Clinical Monitoring Form (CMF), while receiving open naturalistic measurement-based care based on model practice procedures. Patients provided a blood or saliva sample for BDNF val66met genotyping and completed the Childhood Trauma Questionnaire (CTQ). BDNF met allele carrier status, CTQ total score (ranging 25-125, with higher scores indicating greater childhood trauma), and presence or absence of childhood sexual abuse (ascertained from the CTQ-sexual abuse subscale), were evaluated in relation to mean prior-year Clinical Global Impressions-Severity of Illness (CGI-S) score.

Results: 85 patients (44 bipolar I disorder, 36 bipolar II disorder, 5 bipolar disorder not otherwise specified), mean ± SD [median] age 47.3 ± 14.1 [46.7] years, 63.5% female) completed the CTQ. BDNF val66met genotyping was obtained for 80 of these patients. For all 85 patients, who were seen in the clinic on average every 59.0 ± 37.8 (45.6) days, mean ± SD (median) prior-year CGI-S score was 3.1 ± 0.9 (3), CTQ total score was 44.9 ± 16.2 (43), and 30.6% reported a history of childhood sexual abuse. Of the 80 patients with genotype data, 37.5% were BDNF met carriers (7.5% met/met, 30.0% val/met) and 62.5% were non-met carriers (val/val). Among BDNF met carriers but not non-met carriers, individuals with compared to without childhood sexual abuse had significantly higher mean prior-year CGI-S scores (3.5 ± 0.7 versus 2.9 ± 0.7, respectively, $t = -2.4$, $df = 28$, $p = 0.025$), and CTQ total score tended to correlate with mean prior-year CGI-S score ($r = 0.35$, $df = 28$, $p = 0.058$).

Discussion: History of ELS in general, and especially childhood sexual abuse, were associated with greater longitudinal illness severity in bipolar disorder patients who carried the BDNF met allele, consistent with earlier reports of a gene-by-environment interaction between BDNF val66met and ELS with respect to affective outcomes in non-clinical samples. Further studies are warranted to explore the impact of BDNF val66met genotype and ELS on individuals with bipolar disorder.

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98. Decreased Tryptophan Hydroxylase 1 mRNA Expression in PMS/PMDD

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Background: Previous studies suggest that the serotonergic system is implicated in the pathophysiology of premenstrual syndrome (PMS)/premenstrual dysphoric disorder (PMDD). We aimed to investigate the expression of tryptophan hydroxylase 1 (TPH1), the rate-limiting enzyme of serotonin synthesis, in the blood of women with PMS/PMDD.

Methods: 65 women were assessed using the Premenstrual Symptoms Screening Tool (PSST) and 2 months of prospective charting using the Daily Record of Severity of Problems (DRSP). Women were divided into two groups: mild/no PMS ($n = 39$) and moderate to severe PMS/PMDD ($n = 26$). Blood was drawn during the mid-follicular and late-luteal phases of the menstrual cycle, and mRNA expression of TPH1 was measured. Quantitative real-time PCR measured gene expression using primer-probes for TPH1.

Results: In the late-luteal phase, women with moderate to severe PMS/PMDD displayed lower TPH1 mRNA expression than women with mild/no PMS ($p = 0.02$). A significant decrease in TPH1 mRNA expression from the mid-follicular phase to the late-luteal phase was seen among women in the moderate to severe PMS/PMDD group ($p = 0.02$), but not among women with mild/no PMS ($p > 0.05$). Furthermore, in the late-luteal phase, a negative correlation was observed between TPH1 mRNA expression and anger/irritability scores on the PSST, for the moderate to severe PMS/PMDD group only ($r = -0.49$; $p = 0.01$).

Discussion: Our results suggest that decreases in serotonin synthesis may be associated with the pathophysiology of PMS/PMDD and may explain why serotonin-based treatments are effective in this population when administered intermittently (during the late-luteal phase only). Peripheral TPH1 mRNA expression may be a useful biomarker for the diagnosis of PMS/PMDD.

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99. Relationship Between DISC1 SNPs and Brain Volumes in First Episode Schizophrenia

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Background: DISC1 has emerged as a highly plausible candidate gene for both schizophrenia and mood disorders. It codes for a hub protein with an extensive interactome that affects multiple neurodevelopmental processes. Two SNPs, rs66752821 (Leu607Phe) and rs821616 (Cys704Ser), have recently been shown to affect cortical maturation in normal children and adolescents.[1] Phe carriers had a significant attenuation of cortical thinning, while Ser homozygotes had a significant acceleration of cortical thinning. Because schizophrenia is a neurodevelopmental disorder that typically has its prodrome or onset during this time period, and because it has a well-established genetic component, we examined these two DISC1 gene SNPs in relation to brain measures in schizophrenia patients. We wished to explore whether young first episode patients who were neuroleptic naïve or minimally medicated would have show a similar relationship to the recently reported DISC1 genotype results in children and adolescents.

Methods: We studied a sample of 107 young first episode never- or minimally-medicated schizophrenia patients, all of whom were Caucasian. To identify the genotypes we used an Illumina candidate gene chip custom-designed for the study of psychiatric disorders and provided by Janssen Scientific Affairs, LLC. Brain volume measurements were obtained using one of two scanning protocols, which we refer to as MR5 and MR6. Both are multimodal (i.e., acquire T2 or PD sequences in addition to T1), thereby providing optimal discrimination between GM, WM, and CSF. For MR5 subjects each participant's data included T1-, T2-, and PD-weighted images collected on a 1.5-T GE Signa scanner. MR6 was acquired on a 1.5-T Siemens Avanto scanner using T1 and T2 sequences. Brain tissue volumes were obtained using BRAINS software. Data were analyzed using ANCOVA; covariates included MR type, age, sex, intracranial volume, and past antipsychotic exposure measured in doseyears.

Results: In the case of rs6675281, 79 individuals were CC (LeuLeu) homozygotes, while 28 were TC (LeuPhe). Carriers of the Phe allele of rs6675281 were found to have significant increases in multiple gray matter (GM) volumes. In the case of rs821616, 11 individuals were AA (SerSer), while 41 were TA (CysSer), and 55 were TT (CysCys). Ser homozygotes of rs821616 hadsignificant decreases in multiple GM volumes.

Discussion: Case-control studies have shown that patients with schizophrenia have evidence of brain tissue loss at the time of initial presentation with the illness. The losses affect overall cerebral volume as well as both GM and WM; they are most consistently found in the frontal and temporal lobes. Because the tissue losses are present at onset, and because the onset of initial symptoms usually occurs in the teens and twenties, the tissue loss is presumed to be due to disturbances in the neurodevelopmental processes that sculpt the brain into maturity during this time period. The nature of these neurodevelopmental processes is unknown, but pathological pruning is a plausible explanation. Our findings are consistent with this explanation and with the findings of Raznahan *et al.* That is, normal children and adolescents who are carriers of the rs66752821 Phe allele have attenuation of cortical thinning, while rs821616 Ser homozygotes have accelerated thinning. Our findings suggest that young first episode schizophrenia patients may have an intensification of these processes during the prodromal period of their illness and that this may be partially explained by their DISC1 genotype, which contributes to the brain abnormalities observed at the onset of schizophrenia. Raznahan A *et al.* Common functional polymorphisms of DISC1 and cortical maturation in typically developing children and adolescents. *Mol Psychiatry* 2010.

Table 1. Relationship Between DISC1 SNPs and Brain Volumes
rs6675281 (HEP1, Leu607Phe) lsmeans (cc)

	CC(79) LeuLeu	TC (28) LeuPhe	*P*
Cerebral GM	685.33	702.58	.0004
Cortical GM	635.29	650.56	.001
Temporal GM	159.02	162.36	.03
Parietal GM	139.51	143.56	.02
Cerebral WM	456.61	440.26	.03

rs821616 (Cys704Ser)

lsmeans(cc)

	AA(11) SerSer	TA(41) CysSer	TT(55) CysCys	*P*
Cerebral GM	672.06	697.03	688.54	0.004
Cortical GM	621.33	645.81	638.47	0.004
Frontal GM	256.80	268.41	267.92	0.02
Temporal GM	157.47	161.99	158.67	0.03

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100 The Psychosis Spectrum in a Young Community Sample

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Background: Identification of early signs of psychosis in young people can facilitate prevention and early intervention in psychotic disorders. Selection of appropriate samples to screen for neurodevelopmental risk factors has been a major challenge in health care systems, such as the U.S., that lack systematic population based tracking systems. Community screening approaches can enhance the generalizability of findings on neurodevelopmental factors that are associated with increased risk of transition to psychosis (Kaymaz & VanOs, 2010), thereby potentially allowing us to step even further backwards in the time course of illness to define neurodevelopmental trajectories of psychosis. The current investigation aimed to screen for psychosis spectrum symptoms in a large community sample of children and adolescents, to allow subsequent evaluations of the relationships among clinical, neurobehavioral and genetic risk indicators of psychosis.

Methods: Children (age 11-21; n = 5,631; mean age = 15.41; 54% female) participated in the ARRA funded Neurodevelopmental Genomics and Trajectories of Complex Phenotypes (Gur, Hakonarson, PI's), or Grand Opportunity (GO) study that is currently conducting clinical and neurobehavioral phenotypic characterization of a large cohort of 10,000 prospectively accrued community participants, age 8 to 21 years. This 2-year collaboration between the University of Pennsylvania and Children's Hospital of Philadelphia

includes three components: a screen for personal and family history of psychopathology (GOASSESS) and computerized neurocognitive testing of 10,000 participants, and neuroimaging of a subset of 1,000 participants. Psychopathology is assessed using a computerized, structured screener (GOASSESS) that was developed from a modified version of the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS). The psychopathology screener allows symptom and criterion-related assessment of major disorders, including psychosis spectrum, and treatment history. The GOASSESS psychosis section was developed to screen for hallucinations and delusions, their context (substances, illness, medicines), and reported distress/impairment. The PRIME Screen is a 12-item screening questionnaire developed to assess sub-psychotic symptoms (Miller *et al*., 2004). Prior work has shown high sensitivity and specificity in young adult clinical (Miller *et al*., 2004) and non-clinical (college student) samples (Kobayashi *et al*., 2008); these properties combined with its brevity made it especially suitable for incorporation in GOASSESS. To our knowledge, there have been no investigations of the PRIME Screen in a community sample of children and adolescents. We therefore evaluated the PS-R in GO participants. Items are self-rated on a 7-point scale ranging from 0 (Definitely Disagree) to 6 (Definitely Agree). Using the original PRIME Screen, a positive screen is $>=1$ item rated 6. PS-R Total score was also calculated to provide a quantitative risk indicator.
Results: Among 5,631 probands, 6.5% (n = 365; 55% female; mean age = 14.63, s.d. = 2.4) endorsed hallucinations and/or delusions, reportedly occurring outside the context of substance, illness and medicines, and accompanied by significant impairment or distress. The psychotic symptoms reported included hallucinations (by 67.4%) and delusions (by 49.9%) with duration $>=1$ day. Among the remaining participants who denied threshold psychotic symptoms, 11% (n = 635) screened positive on the PRIME screen (mean age = 14.53, s.d. = 2.9; 51% female; PS-R total score = 25.39. s.d. = 11.2).
Discussion: Results of the screen for psychotic symptoms are generally consistent with meta-analytic results by van Os *et al*., (2009) reporting a median prevalence of 5% for psychotic disorders in the general population. The screen for sub-psychotic symptoms yielded a lower percent of screen positives in young participants than in older adolescents (18%, Fresan *et al*.), which could be attributable to different sampling strategies for detecting youth at risk for psychosis. We will evaluate the significance of this discrepancy through follow-up clinical and endophenotype assessment. Ideally, such studies will inform our understanding of the early neurodevelopmental processes associated with developing psychosis in the community. In the future, by refining the predictive ability of endophenotypes, this work ideally will reduce the rate of individuals falsely identified as at-risk in order to develop interventions targeted towards individuals most in need.
Disclosure: M. Calkins: Part 2: University of Pennsylvania. **J. Richard:** None. **K. Merikangas:** None. **M. Burstein:** None. **L. Heaton:** None. **H. Qui:** None. **F. Mentch:** None. **L. Hermannsson:** None. **J. Connolly:** None. **D. Abrams:** None. **R. Chiavacci:** None. **P. Sleiman:** None. **J. Loughead:** None. **R. Gur:** Part 4: Investigator-initiated grants from Pfizer and AstraZeneca, Part 5: NA. **H. Hakonarson:** None. **R. Gur:** Part 4: Pfizer Astra Zeneca.

101. A Map Function Predictive of Risk of Psychosis Obtained by Concurrent Genome Wide Association to Dopaminergic Deficits

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Background: Uncovering the mechanism of inherited risk in schizophrenia has proven challenging despite its high heritability. The use of simplified phenotypes reduces complexity, and common GWAS methods do not address the possibility of uncovering multiple combinations of genotypes (genes) contributing to risk through complex nonlinear interactions.
Methods: We tested the hypothesis that a primary dopaminergic deficit underlies vulnerability to schizophrenia incorporating machine learning and optimization research to solve complex combinatorial problems not approachable with traditional GWAS statistics.
Results: Markers of dopaminergic function and genetic profiles predict the risk status of a cohort drawn from a unique medication-naïve indigenous population. Because of the extreme difficulty of finding neuroleptic naïve patients, we studied a limited sample of 35 subjects with chronic, never-treated schizophrenia, 35 unaffected relatives and 35 matched controls. Blind assessments included motor function, cognitive performance and personality traits. Transcranial ultrasound of the substantia nigra and a genome wide scan were also obtained. We partitioned the sample first based on shared phenotype measures and defined a set of clusters. We then identified groups of participants who shared common genotype clusters. Finally, we combined genetic and phenotypic information in an unbiased fashion and uncovered nonlinear *relations* predictive of risk status (affected, relatives or controls) in a separate sample. The function underlying this prediction is the first demonstration of a map of schizophrenia risk. G enes related to schizophrenia in this sample represent overwhelmingly four molecular networks related to know ontologies.
Discussion: Genetic risk may act primarily by modulating dopaminergic pathway vulnerability to environmental or epigenetic factors.
Disclosure: G. de Erausquin: None. **I. Zwir:** None. **M. Calvó:** None. **N. Florenzano:** None. **G. Gonzalez Aleman:** None. **M. Martínez:** None. **E. Padilla:** None. **M. Breed:** None. **B. Benítez:** None. **H. Belálcazar:** None. **G. Guerrero:** None. **B. Molina Rangeon:** None. **M. Sedó:** None. **H. Conesa:** None. **J. Escobar:** None. **C. Cloninger:** None.

102. Analysis of CpG SNPs in Serotonin System Genes: Analysis of Suicide Attempt in Schizophrenia

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Background: Studies of various serotonin (5-HT) indicators have shown that low levels of serotonergic neurotransmission are associated with suicidal behavior rather than with primary psychiatric diagnosis. Given the above rationale, the primary aims of the current study are: 1) To identify 5-HT SNPs that are associated with suicide attempt in schizophrenia; 2) Create a SNP map in our sample, by selecting SNPs that affect the CpG (methylation) sites in the promoter and in the coding region of 5HT genes.
Methods: We have collected detailed clinical information and DNA samples from 497 schizophrenia patients, allowing us to perform serotonin gene analyses in suicide attempters and non-attempters. Using the structured research interview we determined the presence of suicide attempts lifetime that can be tested using DNA variants in the serotonin system to detect the genetic markers associated with suicide risk in schizophrenia.This cross-sectional DNA sample comprises of 497 subjects with a diagnosis of SCZ, all carefully assessed for lifetime suicide attempt by the means of Beck Scale for Suicide Ideation (BSS). There are 352 men and 145 women with a mean age at the time of the interview of 38.72 ± 10.89 and 147 patients in the sample that have attempted suicide at least once in their lifetime. In the initial step, we will apply a conventional genetic association strategy in order to find any SNP associated with suicide attempt in the schizophrenia. The association study with the 5HT genes (5HT receptors, TPH2, TPH1, SLC6A4) will be performed using the duration of illness as main covariate incorporated in an additive model. A novel mapping analysis will be conducted using a specific bioinformatic tool we have developed, which analyzes only the polymorphic CpG sites in the 5-HT system. This analysis will

looks at the presence or absence of methylation sites affected by the SNP allele. Using this analysis, each subject can have one, two or no methylation sites for each SNP locus, which in turn can be translated in a methylation level of 0, 50 or 100%. This bioinformatic tool can detect the SNPs that are affecting the polymorphic CpG sites throughout the promoter and the coding region of 5HT genes. In this analysis, the SNPs in the candidate genes will be studied under a different perspective considering their direct contribution to the availability of methylation sites within the gene of interest. Furthermore the total number of potential methylation sites at gene level and system level will be calculated. In this abstract the results of this novel analytic technique are summarized. The level of potential methylation was compared using a linear model.
Results: In this abstract the results of this novel analytic technique are summarized.
Among the 108 5HT SNPs selected from the Affy 6.0 only the rs7978482 in the TPH2 gene was significantly associated with suicide attempt ($p = 0.002$).
There were 33 CpG SNPs in the aforementioned panel. The total level of potential metylation in the overall 5HT system (33 SNPs combined) was not associated with suicide attempt ($p = 0.343$) however when considering the potential methylation at the TPH2 locus we found a slight trend ($p = 0.057$) with suicide attempt associated with lower methylation ($x = 0.720\text{-}0.008$).
Discussion: The overall results show no association between 5HT CpG SNPs and suicide attempt however the information of the SNP CpG methylation analysis can be used as covariate in future methylation analysis of 5HT genes. The coverage of the traditional GWAS chips maybe inadequate for experiments aimed to link sequence and methylation variation in the DNA.
Disclosure: V. De Luca: None. **C. Zai:** None. **J. Strauss:** None. **J. Kennedy:** None.

103. Heritability Analyses of Endophenotypic Measures for Schizophreniafrom the Consortium on the Genetics of Schizophrenia (COGS)

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Background: The exploration of the genetic architecture of specific endophenotypes may be a powerful strategy for understanding the genetics of schizophrenia. As part of the Consortium on the Genetics of Schizophrenia (COGS), we have now completed stage 1 of a large multi-site study to characterize the genetic architecture of 12 primary endophenotypes in a large collection of families segregating schizophrenia. Stage 2 of COGS will employ a similar endophenotype strategy in a case-control design.
Methods: Each of the 296 families consisted of a proband with schizophrenia, both parents, and at least one unaffected sibling for a total of 1,286 individuals that have been thoroughly characterized. Twelve primary neurophysiological and neurocognitive endophenotypes were assessed: Prepulse inhibition (PPI), P50 suppression, the antisaccade task, Degraded-Stimulus Continuous Performance Test (DS-CPT) d', Letter-Number Span (LNS) re-ordered, and the California Verbal Learning Task (CVLT-II) total score, as well as Abstraction and Mental Flexibility (ABF), Face Memory (FMEM), Spatial Memory (SMEM), Spatial Processing (SPA), Sensori-motor Dexterity (S-M), and Emotion Recognition (EMO) from the University of Pennsylvania Computerized Neuropsychological Battery (Penn CNB). We have also explored the heritability of secondary measures derived from these endophenotypes. SOLAR was used to estimate the heritability of each endophenotype and secondary measure and to estimate the environmental and genetic correlations between the 12 endophenotypes.
Results: All 12 primary endophenotypes were found to be significantly heritable with heritabilities ranging from 16 to 49%. We also observed significant heritabilities for many of the secondary measures, such as baseline startle reactivity, P50 conditioning amplitude, DS-CPT hit rate, CPT Identical Pairs (CPT-IP) 3-digit d', LNS immediate recall, and CVLT-II delayed recall and semantic clustering. Significant genetic correlations were also observed between many of the 12 endophenotypes, providing some evidence for pleiotropy.
Discussion: As hypothesized, we have found that variation in the 12 primary endophenotypes is indeed quite heritable in schizophrenia families. These also data provide evidence of heritability for several secondary neurophysiological and neurocognitive measures associated with schizophrenia. Obviously no single endophenotype or secondary measure approaches the 80% heritability of schizophrenia itself, since each contributes a portion of the risk for the disorder.These data thus add to the importance of endophenotypes in understanding the genetics of schizophrenia and reflect the complex and inter-dependent nature of both the endophenotypes and of schizophrenia.
Disclosure: T. Greenwood: Part 1: Dr. Greenwood has received unrelated support for consulting services from INFOTECH Soft. **R. Freedman:** Part 1: Dr. Freedman has a patent through the Department of Veterans Affairs on DNA sequences in CHRNA7. **M. Green:** Part 1: Dr. Green has received unrelated support for consulting services from Abbott Laboratories, Dainippon Sumitomo Pharma, Otsuka, Sanofi-Aventis Pharmaceuticals, Takeda, and Teva, and he has been a speaker for Janssen Cilag and Lundbeck. **R. Gur:** Part 3: Dr. Gur has received unrelated research support from Pfizer and AstraZeneca. **K. Nuechterlein:** Part 1: Dr. Nuechterlein has received unrelated support for consulting services from Wyeth/Pfizer, Part 4: Dr. Nuechterlein has received unrelated research support from Ortho-McNeil Janssen Scientific Affairs. **A. Olincy:** Part 4: Dr. Olincy has received unrelated research support from Lundbeck Pharmaceuticals. **A. Radant:** None. **N. Schork:** None. **L. Seidman:** None. **L. Siever:** None. **N. Swerdlow:** Part 4: Dr. Swerdlow has received unrelated compensation for consulting services funding from Neurocrine. **D. Tsuang:** None. **D. Braff:** None.

104. Identification of Functional Variants in Schizophrenia Patients by Next-Generation Sequencing.

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Background: Although association studies have been successful in identifying candidate genes for schizophrenia, few functional loci have been identified. It has been proposed that rare variants of high effect size could be responsible for at least some of the sub-threshold signals observed in GWAS. Next-generation sequencing provides the opportunity to simultaneously scan a large number of candidate genes for rare variants that might contribute to the disease phenotype.
Methods: The sequencing sample comprised a cohort of 160 schizophrenia cases, all self-identified as born in the British Isles. All cases met the Diagnostic and Statistical Manual for Mental Disorders-IV edition (DSM-IV) and International Classification of Diseases 10th edition (ICD-10) criteria for schizophrenia. Diagnosis was made by Operational Criteria Checklist (OPCRIT). The study was approved by both local and multiregional academic ethical committees and each participant provided informed consent. 562 genes were selected for exon focused sequencing, either from the SZgene database on the basis of prior positive association, or from chromosomal regions previously shown to

show association to schizophrenia in GWAS. Sequencing was performed on pooled genomic DNAs (15-16 subjects/pool). Prior to pooling, all samples were quantified by qPCR to ensure balanced contributions to each pool. Exon enrichment was performed with a customized Agilent SureSelect Array (2.434 Mb unique genomic regions). Paired-end sequencing was performed on SOLiD4 (50 bases at F_3 end and 35 bases at F_5 end). Sequences were mapped to the human genome (UCSC.hg18) with BioScope.1.2.1. SNPs were called from the uniquely mapped sequences (5.3 to 9.4 Gb for different pools) with ABI's diBayes (within BioScope.1.2.1.) and in-house scripts with the main parameters (M15Q20n3r2X2Y5): minimum Q-score 15 in the sequence read, putative SNP base Q-score 20, minimum sequence coverage 3 with the putative SNP alternative base, minimum percentage of sequence reads 2% having the putative SNP alternative base, maximum number of 2 SNPs allowed in a sequence read, and maximum percentage of 5% mis-matches allowed in a sequence read. The sequence bases were counted for each pool at each of the combined putative SNP sites. The genic location of the putative SNPs were identified based on refGenes (UCSC hg18). Known human SNPs were identified by comparing with dbSNP (snp131). Functional effects of mis-sense mutations from the putative SNPs were predicted by using PolyPhen2. The alternative allelic frequencies was calculated and compared with those in EUR (CEU + FIN + GBR) populations from 1000 Genomes Project. Although a total 320 alleles were present in the combined pools, a cut off of 250 reads was used to filter the data to avoid excluding true rare variants.

Results: 61% (81,368) of sequencing reads mapped to targeted regions, and 51% (41,467) of these SNPs passed the initial filter cutoff of >250 read counts for inclusion in further analyses. Of the SNPs mapping to untargeted locations only 31% (18,253) would have passed this initial filter, indicating that enrichment was successful. Comparison of allele frequency for known SNPs from the 1,000 genomes project against frequencies determined by sequencing showed a good correlation ($r^2 = 0.78$). A total of 34,264 novel variants were detected in the targeted regions. 5,968 SNPs were determined to be potentially functional: 386 nonsense variants, 1,254 splice site variants (up to 10 from splice site), 186 promoter variants, 3,963 mis-sense variants defined as probably damaging by Polyphen2, and 179 mis-sense variants for which no functional designation was possible.

Discussion: Targeted sequencing of candidate genes in schizophrenic subjects is able to identify large numbers of potentially functional variants. Validation of these variants is required to exclude variants that arise due to sequencing error and other technical artifacts. Once validated, these novel variants will provide to opportunity to investigate their frequencies and effects on pathways thought to be important in the development of schizophrenia.

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105. Further Investigations into the Serotonin Transporter Gene in Schizophrenia: A Family-Based Association Study

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Background: Several lines of evidence suggest that the serotonin transporter (SERT) gene is associated with the pathogenesis of major psychiatric disorders. SERT is known to influence mood, emotion, cognition and efficacy of antidepressants, particularly that of selective serotonin uptake inhibitors. Atypical antipsychotic treatments have been thought to exert some of their effects, at least partially through 5HT receptors. Moreover, recent meta-analysis studies have implicated SERT involvement in schizophrenia illness. Previously we have shown in a family-based association analysis that a three marker haplotype was associated with schizophrenia in Caucasian females. These markers were a 44 bp ins/del variant (5HTT-LPR) which regulates SERT expression, a snp (A/G) within this polymorphic locus, rs25531, shown to alter SERT expression and a third variant, Stin2 in the second intron, known to be a transcriptional enhancer. More recently, a case-control study suggested a three marker haplotype consisting of 5HTT-LPR, Stin2 and rs2066713 variants associated with schizophrenia (Vijayan *et al*., 2009).

Methods: In this expanded investigation, we have tested five new snps in the SERT gene (rs1042173, rs11080121, rs2020942, rs2020937, rs2066713) with risk for schizophrenia in our dataset (CBDB: N = 408 cases) using a "case-parent" trio design. The snp data and the previously reported three markers (5HTT-LPR, Stin2 and rs25531) were analyzed using FBAT for family-based association analysis.

Results: Overall, there were no significant associations observed for any of these eight markers with schizophrenia. However, haplotype analysis suggests preferential co-transmission of risk alleles to the affected female offspring. Significant associations were found for multiple three- and four-marker haplotypes with schizophrenia in Caucasian females only ($p < 0.01$ for 4-marker haplotype: rs11080121-rs2020942-Stin2-rs2020937attaining global significance $p < 0.05$).

Discussion: These results add to prior evidence that that the serotonin transporter gene may play a role in the pathogenesis of schizophrenia, at least in females. Additional analyses are underway to examine the gender-specific risk architecture of this gene. Previous genetic studies have yielded inconsistent evidence for the association of SERT and schizophrenia, perhaps in part because most studies have been unable to control for population stratification in the case-control design and these variants show marked population divergence. The biological and therapeutic evidence suggests that alterations in the function of this SERT protein could be involved in the development of schizophrenia.

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106. Genomewide Association Study Implicates NDST3 in Schizophrenia and Bipolar Disorder

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Background: Schizophrenia and bipolar disorder are major psychiatric disorders with high heritability and overlapping genetic variance, yet few susceptibility loci have been identified and replicated in large-scale studies.

Methods: We performed a genome-wide association study (GWAS) in an ethnically homogeneous cohort of 904 schizophrenia cases and 1640 controls drawn from the Ashkenazi Jewish (AJ) population, and sought to replicate our top result in 10 case-control cohorts (6 schizophrenia and 4 bipolar disorder cohorts containing a total n = 20,446 subjects) drawn from multiple ethnicities. We also examined the relationship between alleles at our top GWAS SNP and expression of a neighboring gene in 119 postmortem cerebellar tissue samples.

Results: In our Ashkenazi GWAS discovery cohort, we identified a genome-wide significant risk locus at chromosome 4q26 (rs11098403, $P = 6.55 \times 10^{-9}$). This SNP demonstrated significant association across the 10 replication cohorts regardless of diagnosis or ethnicity (heterogeneity $I2 = 0$), resulting in a meta-analytic $P = 7.82 \times 10^{-10}$ (OR = 1.12, 95% CI = 1.08-1.16). Additionally, this intergenic SNP was significantly associated with

postmortem cerebellar expression of the neighboring gene NDST3, which encodes an enzyme critical to heparan sulfate binding.
Discussion: Our study suggests a possible role of NDST3 in susceptibility to schizophrenia and bipolar disorder. Heparan sulfate binding is critical to neurite outgrowth, axon formation, and synaptic processes thought to be aberrant in major psychiatric disorders.
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107. Functional polymorphisms of the MC4R and NPY Genes are Associated with Antipsychotic-Induced Body Weight Gain

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Background: The substantial inter-individual variability observed in response and side effects with antipsychotic drugs is likely to largely depend on genetic factors. One of the most debilitating side effects, emerging with many newer antipsychotic drugs, is substantial weight gain associated with cardiovascular complications and metabolic syndrome. We have been dedicating our efforts to investigate genetic causes in the serious side effect of antipsychotic induced weight gain. We have unravelled several important associated variants in the SNAP-25, DRD2, CB1 and CCK genes including clinical and demographical variables in order to assess potential gene x environment factors. Since previous findings by our group and collaborators strongly implicated the leptin-melanocortin energy homeostasis system to be associated with antipsychotic-induced weight gain, we investigated four variants in each of the melanocortin-4 receptor (MC4R) and the neuropeptide Y (NPY) genes in our ongoing studies.
Methods: A total of 237 patients who underwent treatment for chronic schizophrenia or schizoaffective disorder were evaluated for antipsychotic response and induced weight gain for up to six months. The sample consisted mainly of individuals of European descent exposed to clozapine for their first time. We genotyped SNPs rs2229616, rs17782313, rs11872992, rs8087522 in the MC4R gene. As for the NPY gene, SNPs rs16147, rs16475, rs5573 and rs5574 were tested. These latter SNPs were selected based on their functional relevance reported in the literature.
Results: Our analyses with the MC4R gene showed that clozapine treated patients of European ancestry who were carriers of the rs8087522 A-allele (AG + AA) gained on average significantly more weight than non-carriers ($p = 0.027$). Furthermore, our group detected a functional relevance of this by electrophoretic mobility shift assay analyses, where the presence of the A-allele appears to create a transcription factor-binding site.
With respect to the NPY gene, a significant association of rs16147 genotype with weight change was observed in clozapine treated patients of European descent ($n = 54$; $p = .002$). Our preliminary analyses revealed that carriers of the functionally relevant C-allele gained significantly more weight compared to individuals with TT-genotype (TC + CC vs. TT; $5.61\% \pm 5.4$ vs. $0.32\% \pm 4.8$). Similarly, two other polymorphisms (rs5573 and rs5574) were significantly associated with weight change ($p = .009$ and $p = .022$). However, these three associated polymorphisms were found to be in high linkage disequilibrium and thus are unlikely to represent independent findings.
Discussion: Our results tentatively suggest novel associations between functionally relevant markers of the MC4R and NPY genes in patients treated with antipsychotic medication for schizophrenia. These findings will help to create clinical algorithms to identify patients at higher risk for antipsychotic-induced weight gain through personalized medicine. However, replication and further work is needed in order to further understand the role of MC4R and NPY gene variants in antipsychotic-induced weight gain.
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108. Association between a Genetic Marker in HTR2A and Response to the mGlu2/3 agonist LY2140023 Monohydrate in the Treatment of Schizophrenia

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Background: LY2140023 monohydrate (hereafter referred to as LY2140023) is the oral prodrug of LY404039, a selective agonist for metabotropic glutamate 2/3 (mGlu) receptors. LY2140023 is currently under development for the treatment of schizophrenia and has the potential to introduce a novel mechanism of action for antipsychotics. In an effort to identify genetic markers associated with LY2140023 response, two pharmacogenetic studies were conducted – a retrospective analysis based on a 4-week phase-2 proof-of-concept trial H8Y-BD-HBBD [1], and a prospective analysis based on a 24-week phase-2 safety trial H8Y-MC-HBBR.
Methods: In the HBBD genetic study, genetic variants in eight candidate genes were investigated in 193 DNA samples collected from clinical trials HBBD and F1D-US-HGLF. The primary analysis examined the association between genetic variants and change in the Positive and Negative Syndrome Scale (PANSS) total score in Caucasians treated with placebo, LY2140023 or olanzapine using an ANCOVA model under the additive allelic assumption. In the HBBR genetic study of 253 DNA samples, the top findings from the HBBD genetic study were examined in 53 Non-Hispanic Caucasians treated with LY2140023 and 63 Non-Hispanic Caucasians treated with standard of care (SOC) atypical antipsychotics. The genetic association was tested using an MMRM model under the additive allelic assumption at a 0.05 one-sided alpha level.
Results: The HBBD analysis identified 23 single nucleotide polymorphisms (SNPs) that were associated with a change in PANSS total score in response to LY2140023 at Week 4, with the serotonin 2A receptor gene (HTR2A) SNP rs7330461 being the most significant association (unadjusted $p < 0.001$). The T allele of rs7330461 was associated with a larger reduction in the PANSS total score in response to LY2140023 treatment [2]. Consistent with the HBBD results, the T allele of rs7330461 was significantly associated with LY2140023 treatment at Weeks 3 and 4 (one-sided p value < 0.05) in the HBBR analysis. Throughout the 24-week treatment period in HBBR, patients in the T carrier genotype group showed a better treatment response to LY2140023 than those in the A/A genotype group, with statistical significance observed at Weeks 4, 12 and 16 (one-sided $p < 0.05$). Additional analysis indicated that while the T allele was associated with a better response to LY2140023 treatment, this allele was associated with worse response to SOC treatment, with statistical significance observed at Weeks 16 and 20 (one-sided $p < 0.05$).
Discussion: Together, the HBBD and HBBR genetic analyses suggest there is an association between HTR2A SNP rs7330461 and

treatment response to LY2140023 in Non-Hispanic Caucasians. Ongoing studies are focusing on confirmation of these results in larger patient populations as well as the impact of this variant in other ethnicities.

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[2] Liu W, Downing AC, Munsie LM, Chen P, Reed MR, Ruble CL, Landschulz KT, Kinon BJ, Nisenbaum LK, 2010 Pharmacogenetic analysis of the mGlu2/3 agonist LY2140023 monohydrate in the treatment of schizophrenia. *Pharmacogenomics J.* Dec 21. [E-pub ahead of print].

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109. Validation of Candidate Endophenotypes for use in Genomic and Clinical Outcome Studies of Schizophrenia

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Background: One strategy for deconstructing and understanding complex neuropsychiatric disorders such as schizophrenia is to examine discrete, genetically determined endophenotype measures. Endophenotypes are heritable, quantitative, laboratory-based measures that are not apparent to the naked eye and are thought to represent intermediate links in the pathways between genetic variation and the clinical expression of the disorder. The ideal schizophrenia-related endophenotypes exhibit robust deficits in patients, are largely stable over time and across state-related shifts in psychopathology, and are suitable for repeat testing. Unfortunately, many widely used endophenotypes in schizophrenia have not been fully characterized regarding these characteristics, leaving a significant gap in our knowledge for use of these measures in genomic and outcomes research. The objectives of the present study were to characterize the extent to which a battery of several putative schizophrenia-related neurophysiological and neurocognitive endophenotypes are: 1) associated with the illness, stable over 1 year, independent of state-related changes, 3) influenced by potential practice/maturation or differential attrition effects in schizophrenia patients and nonpsychiatric community comparison subjects (NCS). Stability of clinical and functional assessment measures was also assessed.
Methods: A large cohort of clinically stable schizophrenia patients (SZ; n = 353) and nonpsychiatric comparison subjects (NCS; n = 205) completed baseline testing. Of these 558 subjects, 223 (SZ n = 163; NCS n = 58) returned for retesting after 12 months. A battery of neurophysiological (mismatch negativity, P3a, P50 and N100 event-related potential amplitudes and suppression indices, prepulse inhibition and habituation of the acoustic startle reflex, and oculomotor antisaccade), neurocognitive (WRAT-3 Reading, LNS-forward, LNS-reorder, WCST-64, CVLT-II), and functional measures (Global Assessment of Functioning, Scale of Functioning, UCSD Performance-Based Skills Assessment) were administered at intake and repeated at 12 months.
Results: Most neurophysiological and neurocognitive measures exhibited medium to large effect size deficits in schizophrenia, moderate to substantial stability with little evidence of change across the retest interval, and were independent of fluctuations in clinical status. Clinical symptoms and functional measures also exhibited substantial stability over the retest interval.
Discussion: The majority of neurophysiological, neurocognitive, clinical, and even functional measures in a sample of chronic schizophrenia outpatients were highly stable over a 1 year retest interval and do not demonstrate practice or time effects, suggesting that they are suitable as repeated measures for genomic as well as clinical outcome studies. Surprisingly, attrition in the schizophrenia sample was not associated with baseline clinical, cognitive, or functional variables. Factors that may have influenced the present results, including ascertainment biases, are discussed. In this large cohort of schizophrenia patients, the tested endophenotypic measures are stable and hold promise for informing the "gene-to-phene gap" in schizophrenia research.
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110. Structure of Endophenotypes in Schizophrenia: Factor Analysis of 15 Putative Endophenotypes from the Consortium on the Genetics of Schizophrenia (COGS)

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Background: Genetic studies of schizophrenia focus increasingly on putative endophenotypes because their genetic etiology is hypothesized to be simpler than clinical diagnosis, and this presumed simplicity is thought to lie on a more precise path to underlying susceptibility genes than is the more complex diagnosis. The Consortium on the Genetics of Schizophrenia (COGS-1), a multisite family study that collected data between 2003-2009, aims to identify the genetic basis of several endophenotypes. We asked: what is the factor structure of the endophenotypes? How independent are the endophenotypes and what do they have in common?
Methods: We present data with all participants of patient, relatives and control groups who were not missing any of the observed variables from 15 putative endophentotypic measures (schizophrenia probands N = 97, nonpsychotic siblings N = 186, and community comparison subjects (CCS) N = 243). We conducted factor

analyses on the putative endophenotypes including 12 neuropsychological measures of working memory, declarative memory, vigilance, spatial ability, abstract reasoning, face emotion processing, motor speed, as well as3 psychophysiological measures of presumed inhibitory processing including P50, Prepulse inhibition (PPI) and antisaccade (AS) tasks. Our factor extraction method was maximum likelihood, and the rotation method was Varimax with Kaiser Normalization.
Results: Analyses yielded a four-factor solution. Of note, the 3 psychophysiological measures correlated relatively weakly with the factors, with P50 having a negligible factor loading. Only the AS task correlated substantially with the factors, with a maximum loading of .415 on the visual-spatial and abstraction factor. Motor speed was also weakly correlated with a maximum loading of .366. Thus, the four factors were largely cognitive, and they included: 1) visual-spatial and abstract reasoning, 2) memory, 3) processing speed, and 4) working memory.
Discussion: The factor structure of 15 putative endophenotypes was derived from 526 subjects in the COGS-1 dataset. Four cognitive factors, consisting of visual-spatial and abstract reasoning, memory, processing speed, and working memory were extracted, which were largely unrelated to two of the psychophysiological measures (P50 and PPI) or motor measures. The AS task loaded moderately on the visual-perceptual and abstract reasoning factor and thus has a stronger association than do the other psychophysiological measures with higher cognitive functions. This is consistent with a model distinguishing putative endophenotypes on the basis of controlled versus more automatic processing. These data support the use of a battery of endophenotypic measures covering a number of domains. Future analyses will evaluate the similarities and differences between the factor structure in patients, siblings and controls, and measure the association between these factors and genetic data collected in COGS.
Disclosure: L. Seidman: None. **G. Hellemann:** None. **R. Freedman:** None. **R. Gur:** Part 4: Investigator initiated grants from Pfizer and AstraZeneca. **R. Gur:** Part 1: Brain Resource Center (Sydney, Australia), Part 2: none, Part 3: none, Part 4: Investigator initiated grants from Pfizer and AstraZeneca. **G. Light:** Part 1: Consultation: Allergan, Memory, and Roche. **K. Nuechterlein:** Part 1: Consultation: Merck & Wyeth, Part 4: Investigator-initiated research grant from Ortho-McNeill Janssen Scientific Affairs. **A. Olincy:** None. **A. Radant:** None. **L. Siever:** None. **J. Silverman:** None. **N. Swerdlow:** None. **D. Tsuang:** None. **M. Tsuang:** None. **D. Braff:** None. **M. Green:** Part 1: Consultant: Abbott Laboratories, Amgen, Cypress, Lundbeck, and Teva. Speaker: Otsuka and Sunovion.

111. X-linked GABA Receptor Genes show Altered Expression in the Anterior Cingulate Cortex in Schizophrenia

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Background: Schizophrenia is a common and severely debilitating mental illness which is highly heritable and poorly understood. One brain region of interest, the anterior cingulate cortex (ACC), has previously been implicated in the defective regulation of emotion and executive function in schizophrenia. Grey matter reductions in the ACC appear to precede the onset of psychosis in some high-risk individuals, and may progress during the course of the illness. Post-mortem analyses indicate that these changes are accompanied by reduced neuronal, synaptic, and dendritic density in the ACC. A growing body of evidence indicates that the gamma-aminobutyric acid (GABA) system plays an important role in neurodevelopment and this system has been shown to be disrupted in schizophrenia. Therefore we have tested the hypothesis that abnormal expression of GABAergic genes occurs in the ACC in schizophrenia.
Methods: Postmortem subjects diagnosed with schizophrenia and a comparison group of subjects without psychiatric illness were included in this study. RNA was extracted from frozen gray matter from the ACC of 39 schizophrenia cases and 34 control subjects. Quantitative polymerase chain reaction (QPCR) analysis was conducted for thirteen candidate GABAergic genes which were previously found to have altered expression in the prefrontal cortex in schizophrenia or otherwise associated with behavioral correlates of schizophrenia in animal studies. Gene expression data were normalized to the geometric mean of the expression of three housekeeping genes (cyclophylin A, glyceraldehyde-3-phosphate dehydrogenase and beta-glucuronidase) using the relative standard curve method.
Results: Our data indicate interactions between anterior cingulate gene expression of GABA-A receptor alpha 3 (GABAα3) and epsilon (GABAε) subunits and gender on diagnosis. Statistically significant decreases in GABAα3 and GABAε expression levels were detected in male schizophrenia patients relative to a comparison group of male subjects ($p < 0.05$), but not in female schizophrenia patients compared to a female comparison group. Altered expression was not detected in schizophrenia for any of the other GABAergic genes tested.
Discussion: In normal populations, females have a slightly larger ACC than males, but in schizophrenia, this trend is reversed. Both GABAα3 and GABAε are encoded by genes with loci at Xq28, a region previously linked with mood disorders, mental retardation, autism and malformations of the cerebral cortex. Furthermore, the genes encoding GABAα3 and GABAε are considered to be important for brain development, which is considered to be impaired in schizophrenia. Therefore dysfunctions of these genes may contribute to the abnormal development of the ACC in male schizophrenia patients. Although the overall incidence of schizophrenia is similar for men and women, male patients have an earlier age of onset and often have worse clinical outcomes. Identification of predictors of these developmental differences between male and female patients could help elucidate some of the pathophysiological mechanisms giving rise to schizophrenia. Our ongoing studies aim to consolidate these findings.
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112. NMDAR2B Genotype influences Prefrontal mRNA Levels and Working Memory in Schizophrenia and Controls

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Background: Disruption of glutamate neurotransmission through blockade of NMDARs in healthy people produces behavior that resembles negative symptoms and cognitive deficits of schizophrenia and exacerbates symptoms in schizophrenia. NMDAR1 is composed of various subunits with different combinations of 2A-D subunits and an obligatory R1 subunit. NMDAR2A and 2B are abundant in the cortex and 2B is related to neuronal plasticity.
Methods: The impact of NMDAR genotypes and diagnosis (schizophrenia versus control) on mRNA in postmortem prefrontal cortex ($n = 74$) and cognition ($n = 134$) was assessed using allele discrimination assays (4 NMDAR SNPs), qPCR and cognitive assessment (Wechsler Adult Intelligence Scale-III).
Results: In schizophrenia, NMDAR1 mRNA was decreased in prefrontal cortex ($p = 0.01$). NMDAR2B was 43% higher in the left hemisphere in controls ($p = 0.04$) with a significant loss of asymmetrical expression in schizophrenia ($p = 0.01$). Genetic variation in the 3′ UTR of NMDAR2B gene (rs1805502) showed

that minor allele carriers have increased mRNA for the R1 and 2A subunits in controls, but minor allele carriers with schizophrenia have reduced R1 mRNA. The NMDAR2B SNP rs1805502 minor allele was also associated with significantly lower Digit Symbol Substitution ($F = 4.03$, $p = 0.047$) and Arithmetic scores ($F = 5.9$, $p = 0.016$) in schizophrenia.
Discussion: These results provide the first evidence that genetic variation in NMDAR2B may be related to changes in NMDAR subunit composition via altered transcription levels. This is also the first report showing that the minor allele of the NMDAR2B SNP rs1805502 is associated with significantly reduced prefrontal NMDAR1 mRNA and reduced working memory performance in schizophrenia.
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113. Genome-Wide Analysis of Antipsychotic Drug Response in Schizophrenia

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Background: The study was to identify genetic variants affecting the individual differences in response to antipsychotic treatment. We have performed a genome-wide analysisto search for SNPs associated with change in PNASS ratings (positive syndrome, negative syndrome and general psychopathology) after medications and risk for discontinuing use of medication. We incorporated plasma clearance data into our GWAS model which has not previously been considered in GWAS studies of this dataset.
Methods: The study was based on the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study, a clinical trial with up to 18 months of follow up during medication to compare the effectiveness of antipsychotic drugs. Clinical and genotype data was from the National Institute of Mental Health repository (www.nimhgenetics.org). Outcome analysis was based on change (from the baseline to last evaluation on medication) in PANSS ratings. Top associated SNPs were further tested in the same sample, using another outcome measure, the time to discontinuing use of medication, which was the primary outcome in the CATIE study. SNPs showed significant association with time to discontinuation were also examined for associations with the reasons for discontinuations (e.g. ineffective therapeutic effect, side effect and patient's decision). 411 Caucasian subjects who were assigned for a medication during the first phase of the CATIE trial, and who had at least two PANSS ratings were included for analysis. Drug clearance was calculated based on a population model as described previously. A general linear model and Cox proportional hazard model were used to analyze the data while controlling for drug clearance, type of medications, and other demographic variables of patients.
Results: A single nucleotide polymorphism in *XKR4* showed the most significant association with change in positive syndrome ($p = 8.23\text{-}e08$) and in general psychopathology ($p = 8.77e\text{-}07$), but nominal significant association with negative syndrome ($p = 1.0e\text{-}02$). *XKR4* was implicated previously in GWAS study of response to the antipsychotic iloperidone in schizophrenia patients and has been associated with ADHD in a follow-up study of previous top GWAS signals. However, this SNP did not predict time to discontinuation. One SNP in *FMOD* ($p = 0.0017$) was significantly associated with risk for discontinuing use of medication, and four other SNPs in *NDRG1, GNA4, GHITM and C2CD2* showed trends for association (0.05, one-sided) with time to discontinuing use of medication for all reasons. We failed to find any significant associations of these 5 SNPs with any specific reason for discontinuing use of medication, likely due to limited sample size.
Discussion: Through genome-wide analysis incorporating drug clearance data, we provide evidence for that *XKR4* is associated with response to antipsychotic treatment measured by the change in PANSS ratings, and found that five SNPs in other genes were also associated with both the change in PANSS ratings and the time to discontinuing use of medication.
Disclosure: F. Zhang: None. **K. Bigos:** None. **D. Weinberger:** None.

114. Reduced Anterior Cingulate Glutamate in Euthymic, but not Depressed, Patients with Obsessive Compulsive Disorder.

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Background: Obsessive-compulsive disorder (OCD) is characterized by hyperactivity in a network of forebrain structures, including the anterior cingulate cortex (ACC). Convergent evidence suggests that dysregulation of the excitatory amino acid neurotransmitter glutamate may contribute to the disorder. Dysregulation of glutamatergic neurotransmission has also been described in major depressive disorder, which is often comorbid with OCD; potential interactive effects between the two diagnoses' effects on neurotransmitter levels remain largely unexamined.
Methods: We examined ACC glutamate and other neurochemicals in 22 adult OCD patients (11 with comorbid depression; 11 medication free) and 12 controls, using ^{1}H MRS at 4 Tesla. OCD symptoms, depression, and anxiety were assessed.
Results: ACC glutamate did not differ significantly between OCD patients, taken as a group, and controls. However, when OCD patients were subdivided by the presence or absence of comorbid depression, those with low levels of depressive symptoms had significantly lower glutamate levels; patients with comorbid depression showed ACC glutamate levels indistinguishable from controls. Within the OCD group, ACC glutamate correlated with clinical measures of depression and anxiety; the correlation with anxiety was the most robust.
Discussion: Our results reveal unexpected heterogeneity in ACC glutamate in patients with OCD, with OCD diagnosis interacting with depressive and anxiety symptomatology such that only non-depressed, low anxiety patients exhibit a clear abnormality in glutamate. This heterogeneity among OCD patients may explain some inconsistency among previous MRS reports of glutamate in OCD and suggests that reduced ACC glutamate may be a biomarker of a clinically distinct subset of OCD patients.
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115. Effects of Rapid Tryptophan Depletion on Reactive Aggression in Adults with Attention-Deficit/Hyperactivity Disorder (ADHD)

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Background: Recent research has shown that symptoms related to attention-deficit hyperactivity disorder (ADHD), a disorder

predominantly linked to changes catecholaminergic neurotransmission, are also subject to variation of serotonin-related genes. Aggressive behavior often occurs co-morbid to ADHD, in particular in younger ages (i.e. conduct disorder). As regards the neurobiology of aggressive behavior, the neurotransmitter serotonin (5-HT) was shown to be involved, with evidence coming from animal studies and studies in adults. However, to date there are no studies investigating the effects of a diminished central nervous 5-HT synthesis rate on reactive aggression in adult patients with ADHD, which was the aim of the present study. We investigated the effects of rapid tryptophan depletion (RTD) and following diminished central nervous 5-HT synthesis on reactive aggression in adults with ADHD, a disorder known to be associated with aggression and impulsivity.
Methods: Twenty male adult patients with a confirmed diagnosis of ADHD and twenty male healthy adult controls were recruited from various university and non-university backgrounds. The study was carried out using a double-blind within-subject cross-over design. Administration of RTD and a tryptophan (TRP) balanced amino acid load (BAL, sham depletion) as a control condition was randomized and counterbalanced. On one day subjects received an amino acid drink lacking TRP (RTD condition) in accordance with Moja and colleagues (Moja *et al.*, 1988) which was modified for the impact of body weight (Zepf *et al.*, 2008). On a further day they received BAL. Testing days were spaced seven days apart. In both groups ten participants received the RTD drink on the first day of the study and ten participants on the second. Both, the patients and the investigator were blind to the order of administration RTD/BAL. On each testing day 3.25 hours after administration of RTD/BAL (time point of approx. 90% reduction in TRP uptake), patients were administered the Point Subtraction Aggression Game (PSAG), a task designed to provoke reactive aggressive behavior involving low (LP) and high provocation (HP) conditions. In this task subjects competed with a fictitious opponent of the same gender on points. Subjects were told that they would be playing against a person who was sitting in a different room of the building, and that the computers were linked via a local area network (LAN). The investigator acted as if he would call the mentioned person by phone clarifying relevant details for playing the game via LAN such as the subject's identity number. Baseline impulsivity measures were assessed using the Barratt Impulsiveness Scale (BIS).
Results: A 2 (group: ADHD vs. controls) x 2 (treatment: RTD vs. BAL) x 2 (provocation: HP vs. LP) RMANOVA showed a significant group-by-treatment interaction (p_{adj} = 0.000, F = 6.256, df = 1, 39). Additional post-hoc analyses indicated that in the group of patients fewer points were subtracted for the LP condition under RTD when compared to BAL (t = 2.341, p = 0.03, df = 1, 20), for the HP condition no such difference was detected (t = 1.241, p = 0.2297, df = 1, 20). In the group of healthy controls more points were subtracted for the LP condition under RTD when compared to BAL (t = 2.382, p = 0.0278, df = 1, 20). For the HP condition no such difference was found (t = 1.318, p = 0.2031, df = 1, 20). Participants in both groups subtracted more points for the HP condition when compared to LP (p_{adj} = 0.000, F = 49.551, df = 1, 39). In the group of patients there was a significant negative correlation between the RTD-effect (point subtraction RTD minus BAL) after LP (Delta-LP) and baseline impulsivity scores as indexed by the BIS sub-scale motor impulsivity (MI; *Rho* = -0.506; p_{adj} = 0.046), with MI explaining approximately 25.6% of the shared intra-individual variance. Delta-LP was also negatively correlated with the BIS total impulsivity scale (*Rho* = -0.448; p_{adj} = 0.048; explaining approximately 20.1% of the shared intraindividual variance). In the control group no such correlations were found for Delta-LP and MI (*Rho* = -0.188, p = n.s.).
Discussion: This is the first study to investigate the effects of RTD on reactive aggression in adult patients with ADHD in comparison to healthy controls. In the subgroup of patients with ADHD there was a negative correlation between trait-impulsivity scores and Delta-LP, which is in line with data obtained in children and adolescents with ADHD (Zepf *et al.*, 2008). Together with previous findings the data provide evidence for an inverse relationship between trait-impulsivity and the RTD-effect on reactive aggression in patients with ADHD, and that this relationship can be found in both adolescence and adulthood.
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116. CRP is Associated with Physical Well-Being, but not with Early Life Stress or Depression Symptoms, in Healthy Adults

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Background: C-reactive protein (CRP) is an acute phase protein produced by the liver in response to infection or injury. It has become known as a marker of chronic, low-grade inflammation and is widely recognized as a risk marker for cardiovascular disease. More recently, CRP has been associated with a growing number of other conditions, including major depressive disorder, generalized anxiety, and obesity. Despite their diverse manifestations, these conditions may be linked by chronic inflammation. However, it is unclear whether markers such as CRP are an epiphenomenon or if CRP plays a direct role in the development of inflammation-induced morbidity. CRP has also been associated with early life stress.
Methods: We examined the relationship between plasma CRP and self-reported measures of mental and physical health in 92 healthy adults without psychiatric or major medical disorders. Adults were excluded if they met criteria for major (lifetime) Axis I psychaitric disorder on SCID or evidenced medical disorders on review of past health history, physical examination, review of organ systems, or on results of a panel of laboratory tests. Anthropometric measurements were done to determine body mass index (BMI), and selected self-report assessments were used to evaluate somatic and mental health domains.
Results: CRP was significantly correlated with body mass index (BMI; r = 0.477, p < 0.001), but it was not significantly linked to gender or any measures of early life stress. State anxiety (r = 0.218, p = 0.037), but not depression symptoms, was positively correlated with CRP. Nonspecific pain, fatigue, and inferior overall quality of physical health were associated with higher CRP concentrations in our sample (all p < .05 or p < .01), even after controlling for effect of BMI.
Discussion: Self-ratings of inferior overall physical well-being, but not depressive symptoms or early life stress, were significantly related to plasma CRP in healthy adults. Nonspecific pain, fatigue, and diminished quality of overall physical state were correlated with higher plasma concentrations of CRP, perhaps reflecting early

manifestations of future disorders. Elevated CRP did not appear to be a consequence of early life stress among healthy adults who were selected for the absence of mood and other psychiatric disorders.
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117. Proinflammatory Cytokines in Teenage Suicide Brain

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Background: There are several studies that suggest a relationship between the immune system and the pathophysiology of depressive illness. This is based on the observations that many of the proinflammatory cytokines are increased in the plasma of depressed patients and that administration of certain cytokines, such as interferons, produce depression-like symptoms in patients with chronic hepatitis or other forms of cancer. Stressful events have also been shown to cause changes in the immune function. A report suggests that exposure to stressful life events causes impairment in various aspects of cellular immune function. Since both depression and stress are major risk factors for suicidal behavior, this observation may suggest a role for altered immune function in suicide and suicidal behavior. Although the role of cytokines and immune dysregulation has not been studied in great details in suicide, there is some direct and indirect evidence suggesting a relationship between immune dysregulation and suicide, as some investigators have found increased microgliosis in the postmortem brain of suicide victims. Alterations in the immune function or in the proinflammatory cytokines have been observed in the plasma or serum of depressed patients. However, it is not clear if there are similar changes in the brain of subjects with depression or suicide. To examine the role of proinflammatory cytokines, we determined the protein and mRNA expression of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α in the postmortem brain obtained from teenage suicide victims and normal control subjects.
Methods: The postmortem brain samples were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, MD, USA. Samples were obtained from 24 teenage suicide victims and 24 normal teenage control subjects. Psychological autopsy was performed and the subjects were diagnosed according to the Schedule for Clinical Interviews for DSM-IV (SCID). The brain samples were stored at −80°C till assayed. Determination of Cytokine Levels The protein expression levels of IL-1β, IL-6 and TNF-α were determined in the cytosol fraction by enzyme-linked immunosorbent assay (ELISA) using commercially available Quantakine® kits for human IL-1β, human IL-6 and human TNF-α purchased of R&D Systems, Minneapolis, Minnesota. The gene expression of IL-1β, IL-6 and TNF-α were determined using the real-time RT-polymerase chain reaction (qPCR) technique. Statistical differences in age, postmortem interval (PMI) and cytokine levels between normal controls and suicide victims were evaluated by student t-test.
Results: There were no differences in the mean age (the age range was between 13 and 20 years) between teenage suicide victims and normal controls. When we compared the protein expression levels of the proinflammatory cytokines we found that the protein expression levels of IL-1β and TNF-α were significantly increased in teenage suicide victims ($n = 24$) compared with normal control subjects ($n = 24$). The levels of IL-6 were also higher in teenage suicide victims compared with normal control subjects at a p level of 0.06. When we compared the mRNA levels of proinflammatory cytokines, we found that the mRNA levels of IL-1β, IL-6 and TNF-α were significantly increased in the prefrontal cortex (PFC) of teenage suicide victims ($n = 24$) compared with normal control subjects ($n = 24$).
Discussion: This study thus indicates that the levels of proinflammatory cytokines IL-1β, IL-6 and TNF-α are significantly increased in the PFC of teenage suicide victims compared with normal control subjects. Although a relationship between altered immune system and suicide has been suggested, only a few studies have determined the levels of proinflammatory cytokines in the brain of depressed or suicidal subjects. The proinflammatory cytokines may produce their physiological and behavioral effects through various mechanisms; one of the mechanisms suggested is their interaction with the hypothalamic-pituitary-adrenal (HPA) axis and the neuroendocrine system. Since a dysregulation of the HPA axis has been implicated in suicidal behavior, our study may suggest that these abnormalities may also be related to an abnormality of the immune function, as evidenced by increased proinflammatory cytokines in the suicide brain. The observed increase in the proinflammatory cytokines in the suicide brain may suggest that the inflammatory cytokines could be a useful target for developing therapeutic agents for treatment of suicidal behavior.
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118. Vipri Gene Expression is Increased in the Post-Mortem Hippocampus of Individuals with Major Depressive Disorder

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Background: Single nucleotide polymorphisms in circadian genes have been associated with mood disorders. For example, VIP and CLOCK were found to be associated with Bipolar Disorder, whereas CRY1 and NPAS2 were associated with Major Depressive Disorder (MDD).
Methods: We used mRNA *in situ* hybridization to assess gene expression patterns of five circadian genes (CLOCK, VIP, VIPR1, NPAS2 and ADCYAP1). We analyzed the post-mortem human hippocampus of 14 controls (13 males, 1 female) and 21 individuals with MDD (13 males and 8 females).
Results: General expression patterns were as follows from high to low: CLOCK (DG, CA3, CA2, CA1), ADCYAP1 (DG, CA3 = CA2, CA1), NPAS2 (DG, CA2, CA3, CA1) and VIPR1 (DG only). When collapsed across levels of the dentate gyrus of the hippocampus, VIPR1 was increased in MDD compared to controls. We are,

currently, analyzing VIP gene expression by mRNA *in situ* hybridization. There was also a nonsignificant trend for CLOCK to be increased in the dentate gyrus in MDD compared to controls. NPAS2 and ADCYAP1 were not significantly different between the groups. Since there was only one control female, we could not statistically analyze for a gender effect in controls. We could, however, assess gender differences within MDD. There was a nonsignificant trend for a gender effect within MDD for CLOCK, with depressed males exhibiting less CLOCK expression than depressed females. There were no gender differences in MDD for VIPR1.

Discussion: In summary, we found altered gene expression in VIPR1 in the hippocampus of individuals with MDD. Therefore, drugs that target the VIP system may be effective antidepressants.

Disclosure: C. Turner: Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **W. Bunney:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **A. Schatzberg:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **J. Barchas:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **R. Myers:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **E. Jones:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **H. Akil:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″. **S. Watson, Jr.:** Part 4: ″The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. A shared intellectual property agreement exists between this philanthropic fund and the University of Michigan, Stanford University, the Weill Medical College of Cornell University, HudsonAlpha Institute of Biotechnology, the Universities of California at Davis, and at Irvine, to encourage the development of appropriate findings for research and clinical applications″.

119. Cerebrospinal Fluid Substance P-Like Immunoreactivity: Correlates with Aggression in Personality Disordered Subjects

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Background: Neurochemical studies have pointed to a modulatory role in human aggression for a variety of number of central neurotransmitters; some (e.g., serotonin) appear to play an inhibitory role, while others (e.g. vasopressin) a facilitator role in the modulation of aggression. While recent animal studies of Substance P have suggested a facilitator role for Substance P in the modulation of aggression no human studies of Substance P have yet been reported regarding aggression.

Methods: Basal lumbar cerebrospinal fluid (CSF) was obtained from 38 physically healthy subjects with Personality Disorder (PD) and were assessed for CSF Substance P-Like Immunoreactivity (CSF SP-LI) and were correlated with measures of aggression and impulsivity. Aggression measures included the Aggression score from the Life History of Aggression assessment (LHA) and the Aggression Factor score from the Buss-Durkee Hostility Inventory (BDHI). Impulsivity measures included the Impulsiveness Scale from the Eysenck Personality Questionnaire II (EPQ-II) and the Barratt Impulsiveness Scale-Version 11 (BIS-11). Composite variables for "aggression" and "impulsivity", were constructed by taking the average of each subject's z-scores for the primary behavioral measures. CSF SP-LI was determined in blinded CSF samples by solid phase radioimmunoassay (RIA) using a highly specific substance P antibody. The sensitivity of the assay was 6 fmol/ml. Intra-assay variability was 6.5% and inter-assay variability was 9.4%.

Results: In all subjects, CSF SP-LI was significantly, and directly, correlated with composite aggression ($r_s = .33$, $p < .05$) but not composite impulsivity ($r_s = .19$, $p = .27$). Within composite aggression, CSF SP-LI was significantly correlated with the BDHI ($r_s = .39$, $p = .015$), but not with the LHA ($r_s = .19$, $p = .27$), Aggression measure. Within the BDHI Aggression variable, CSF SP-LI was significantly correlated with both Verbal ($r_s = .39$, $p < .02$) and Direct Physical ($r_s = .33$, $p < .05$) BDHI Assault variables. CSF SP-LI did not differ as a function of history of prior suicide attempt [SA + ($n = 9$): 35.4 ± 13.8 fmol/l *vs.* SA- ($n = 29$): 31.8 ± 10.8 fmol/l; Mann-Whitney $U = 115$, $p = .60$). There was no difference in CSF SP-LI as a function of current or life history of Axis I conditions and it did not correlate significantly with non-aggressive, personality dimensions such as Neuroticism, Psychoti-

cism or Extraversion or Novelty-Seeking, Reward Dependence or Harm Avoidance.
Discussion: These data suggest a direct relationship between CSF SP-LI concentration and measures of aggression in human subjects. This adds to the complex picture of the central neuromodulatory role of impulsive aggression in human subjects.
Disclosure: E. Coccaro: Part 1: Scientific Advisory Board, Azivan Pharmaceuticals, Inc., Part 2: < $10,000 per year. **M. Owens:** Part 1: Research Grants: NIH, Eli Lilly, Lundbeck A/S, Cyberonics, Ortho-McNeil Janssen, AstraZeneca, Dainippon Sumitomo Pharma, SK Life Sciences, Sunovion Pharmaceuticals., Part 2: RJ Reynolds -2011, Part 4: Eli Lilly, Lundbeck A/S, Cyberonics, Ortho-McNeil Janssen, AstraZeneca, Dainippon Sumitomo Pharma, SK Life Sciences, Sunovion Pharmaceuticals., Part 5: No. **B. Kinkead:** None. **C. Nemeroff:** Part 1: Consulting: Xhale, Takeda Stockholder: CeNeRx BioPharma, NovaDel Pharma, Inc., PharmaNeuroBoost, Revaax Pharma, Xhale Other Financial Interests: CeNeRx BioPharma, PharmaNeuroBoost Patents: Method and devices for transdermal delivery of lithium (US 6,375,990B1): Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by vivo assay (US 7,148,027B2) Scientific Advisory Boards: American Foundation for Suicide Prevention (AFSP), CeNeRx BioPharma, National Alliance for Research on Schizophrenia and Depression (NARSAD), NovaDel Pharma, Inc., PharmaNeuroBoost, Anxiety Disorders Association of America (ADAA) Board of Directors: AFSP, NovaDel Pharma, Inc., Part 2: CeNeRx Pharmaneuroboost American Psychiatric Publishing NovaDel Pharma Xhale AstraZeneca, Part 4: Agency for Healthcare Research and Quality (AHRQ).

120. Hippocampal NAA and Volume: Potential Response Biomarkers to Glutamate-Based Drugs

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Background: N-Acetylaspartate (NAA) is a putative marker of neuronal plasticity that has been investigated as a treatment response biomarker across several neuropsychiatric disorders. Following 8 weeks of treatment with riluzole, a glutamate reuptake enhancer and release inhibitor, in patients with generalized anxiety disorder, we reported differential changes in hippocampal NAA concentrations, which covaried by the degree of therapeutic benefit. In this study, we investigated associations between hippocampal volumetrics, hippocampal NAA, and response to riluzole.
Methods: Eighteen medication-free patients with chronic GAD of moderate severity received 8 weeks of open-label riluzole. Eight matched healthy subjects (HS) were enrolled as control group. 12 GAD and 8 HS were submitted to high-resolution structural magnetic resonance imaging (MRI) along with proton MR spectroscopy (MRSI) at baseline and at the end of the treatment period. Patients were identified as high-responders (n = 7) or low-responders (n = 5), based on week 8 Hamilton Anxiety Rating Scale (HAM-A) 7.
Results: GAD patients had significantly smaller hippocampal volumes compared to HS [Cohen's $d = 1$, $p = 0.04$]. Total hippocampal volume differences, from baseline to week 8, positively correlated with NAA concentration changes in GAD [$r_s = 0.62$, $p = 0.04$] but not in HS. This correlation was more profound in the right hippocampus in GAD [$r = 0.81$, $p = 0.002$], but not in HS [$r = -0.38$, $p = -0.39$; Fisher r-to-z $p = 0.017$]. In GAD patients, change in hippocampal volume was positively associated with improvement in HAMA-A [$r_s = 0.62$, $p = 0.03$]. Pre-treatment, there was a main effect of response status, with high-responders having smaller hippocampus compared to low-responders and HS [$F(2,17) = 5.85$, $\eta^2 = 0.41$, $p = 0.01$]. Exploratory cortical thickness analyses showed widespread cortical differences among groups.
Discussion: The strong associations between hippocampal volume, NAA, and response to riluzole suggest that NAA has potential utility as a mechanistic biomarker to assess response to glutamate modulating agents. Moreover, the lower volume of hippocampus in the high-responders group is consistent with the hypothesis that heightened pretreatment glutamatergic function moderates response to riluzole. These pilot data support hippocampal volume and NAA as response biomarkers in patients treated with a glutamate-modulating agent, a finding that warrants replication in an expanded sample.
Disclosure: C. Abdallah: None. **J. Coplan:** Part 1: JDC receives grant support from GlaxoSmithKline, Pfizer, and Alexza Pharmaceuticals. He is on the Pfizer advisory board and gives talks for BMS, AstraZeneca, GSK, and Pfizer., Part 2: BMS, AstraZeneca, GSK, and Pfizer., Part 3: BMS, AstraZeneca, GSK, and Pfizer., Part 4: GlaxoSmithKline, Pfizer, and Alexza Pharmaceuticals., Part 5: Not applicable. **A. Jackowski:** None. **X. Mao:** None. **D. Shungu:** None. **S. Mathew:** Part 1: Grant Support: NIMH, NARSAD, Dept. of Veterans Affairs. Consultant (last 24 months): Cephalon, Roche, Noven, AstraZeneca., Part 2: Cephalon, Roche, Noven, AstraZeneca., Part 3: Cephalon, Roche, Noven, AstraZeneca., Part 4: NIMH, NARSAD, Dept. of Veterans Affairs.

121. Predictors and Consequences of Cognitive Therapy for PTSD: Neural Responses to Emotional Anticipation

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Background: Intimate partner violence (IPV) is one of the most common causes of posttraumatic stress disorder (PTSD) in women. The few studies that have investigated changes in brain response after cognitive behavioral therapy (CBT) for PTSD have reported decreased amygdala and increased anterior cingulate cortex (ACC) response during emotional processing (Felmingham *et al.*, 2007; Roy *et al.*, 2010). Using a backward masking paradigm in which emotional faces were presented subconsciously to PTSD patients, Bryant *et al.*, (2008) reported that greater amygdala and ACC activation pre-treatment was predictive of worse treatment response to CBT. However, it has been theorized the directional relationship between prefrontal activation and treatment outcome may depend on whether emotional stimuli are processed consciously or subconsciously. The current study utilized an anticipation paradigm involving conscious processing of emotional stimuli to test the following hypotheses: a) cognitive therapy for IPV-PTSD would lead to reduced activation in amygdala and insula regions and enhanced activation of prefrontal and ACC regions and b) greater ACC response would relate to better treatment response.
Methods: A total of 14 PTSD patients (mean age = 40.07, SD = 7.44, all female) completed the emotional anticipation task during functional magnetic resonance imaging (fMRI) before and after Cognitive Trauma Therapy for Battered Women (CTT-BW). All subjects completed the Clinician Administered PTSD Scale (CAPS; M = 70.57, SD = 17.58) before and after treatment. The anticipation task, which involves cued anticipation of positive and negative affective images, was conducted during one BOLD scan collected using a 3-Tesla scanner. The primary regressors of interest included anticipation of negative (ANI) and

positive (API) images. T-tests were conducted to examine task effects (ANI-API) on activation at pre-treatment, while an analysis of variance was performed to identify condition (ANI vs. API) by time (pre vs. post treatment) interaction effects on voxel-wise percent signal change. Huber robust regressions were conducted to examine the relationship between pre-treatment activations during anticipation of negative images and normalized post-treatment CAPS score (covarying for pre-treatment CAPS score). Activations were considered significant at $p < .05$, corrected for multiple comparisons using Monte Carlo methods for whole-brain analysis. Discussion of results focus on the following *a priori* regions of interest: bilateral insula, amygdala, medial prefrontal cortex (PFC), anterior and posterior cingulate, and dorsolateral PFC.

Results: CTT-BW treatment significantly decreased PTSD symptoms as measured by the CAPS (Pre-treatment, Mean = 66.07, SD = 16.78; Post-treatment, Mean = 16.29, SD = 16.81; $p < .001$). At pre-treatment, the anticipation task elicited responses within the right anterior insula, for which activation was greater during anticipation of negative than positive images, and within the bilateral posterior insula, left posterior cingulate, and medial PFC and ACC (BA 32, 10), for which activation was greater for anticipation of positive than negative images. Treatment was found to significantly influence activation within the right anterior insula, for which activation during negative anticipation decreased, and posterior cingulate, dorsal ACC, and dorsolateral PFC, for which activation during negative anticipation increased from pre to post treatment. Further, robust regression analyses revealed that pre-treatment responses within the dorsal (BA 32) and posterior (BA 31) cingulate during anticipation of negative images was negatively related to post-treatment CAPS score, while activation within the right anterior insula was positively related to post-treatment CAPS score.

Discussion: Results suggest that CTT-BW is effective at reducing symptoms of PTSD and is associated with decreased activation within brain regions associated with emotional anticipation and interoception (anterior insula) and increased activation in brain regions involved in conflict monitoring and cognitive regulation of emotion (ACC and dlPFC). Further, results suggest that the more PTSD patients are able to engage dorsal and posterior cingulate regions when preparing for consciously-processed negative events, the more they may benefit from cognitive-based therapies, whereas proneness towards activating the anterior insula during emotional anticipation may represent a barrier to responding fully to such treatments.

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122. Neural Dysfunction when Appraising Threat during Extinction Recall: Effects of Anxiety and Development

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Background: Childhood anxiety disorders signal increased risk for various mental illnesses. Nevertheless, many anxious children mature to become adults without signs of psychopathology. Understanding the interaction among attention, threat appraisal, and fear learning may help identify subgroups of anxious children most likely to develop anxiety. A "screaming lady" paradigm was explicitly designed for developmental research on fear conditioning (Lau *et al*., 2008) and extinction recall (Britton *et al*.,, 2010). In this study, we examined anxiety-related and developmental effects on subjective and neural correlates during extinction recall in adults and youth with and without anxiety disorders.

Methods: A total of 143 individuals participated in fear conditioning and extinction procedures in the psychophysiology laboratory. From this total, 18 anxious adults, 23 anxious youth, 31 healthy adults, and 42 healthy youth completed the procedures. During fear conditioning, two pictures of female faces displaying neutral expressions served as conditioned stimuli (CS + and CS-) and a "screaming lady" served as the unconditioned stimulus (US). During extinction, the CS + and CS- were presented in the absence of the US. 15 anxious adults, 14 anxious youth, 28 healthy adults, and 25 healthy youth completed extinction recall in a 3T MRI scanner. During extinction recall, individuals viewed morphed images continuously varying in similarity from the CS- to CS + . Individuals reported whether the CS screamed in the past (explicit memory) or whether they were afraid (threat appraisal). Pre-processing and whole-brain, voxel-wise, random-effects analyses were conducted in AFNI. Diagnosis x age group x attention state x quadratic trends across morphed images were tested using linear mixed models using $p < 0.05$ corrected threshold. This four-way interaction was decomposed after extracting data from functionally-defined regions of interest.

Results: Patients and younger individuals (22 anxious youth: 10.2 ± 1.6 years, 6 males, 7 healthy youth: 10.7 ± 2.3 years, 3 males) were more likely to voluntarily discontinue participation [both $p < 0.01$]. Overall, both youth and adult patients rated more anxiety to the conditioned stimuli during fear conditioning and extinction than the healthy groups [$p < 0.001$]. During fear conditioning, individuals rated more anxiety in response to the CS + compared to the CS- [$p < 0.001$]; however, no interactions with diagnosis or age group were noted [all $p > 0.5$]. During extinction recall, behavioral data indicate a quadratic pattern in the responses to morph images [$p < 0.001$]. This quadratic trend is more dramatic in the explicit memory condition than the threat appraisal condition [$p < 0.01$]. No group differences in behavioral data were detected [all $p > 0.2$]. Whole-brain analyses indicate four-way interactions (diagnosis x age group x attention x quadratic trend) in two regions, the subgenual anterior cingulate (sgACC, [-9, -26, -9]) and the ventromedial prefrontal cortex (vmPFC, [4, -49, -6]). During threat appraisal, both patient groups exhibited less sgACC activation than their respective healthy peer groups [$p < 0.007$]. This difference was a main effect and no significant differences in the quadratic response were noted [all $p > 0.6$]. In the vmPFC, patient youth had a greater quadratic trend across the morphs compared to patient adults and healthy youth [both $p < 0.02$].

Discussion: During extinction recall, a gradient between "safety" and "threat" was detected. When appraising threat, anxiety-related dysfunction was detected in the sgACC, the human homologue of infralimbic cortex and this dysfunction did not vary across morphed images. These results suggest that this anxiety-related dysfunction may appear early and persist into adulthood. Patterns within the vmPFC indicate an interaction between diagnosis and age group, suggesting maturation of the system. Deviations from the normal maturation trajectory of vmPFC function and threat-safety classification ability may allow the identification of sensitive periods for clinical expression of anxiety disorders.

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123. Shared Pathophysiology in the Pediatric Anxiety Disorders: Conflict-Related Hyperactivation of the Anterior Cingulate Cortex

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Background: Failure to inhibit pre-potent, but contextually inappropriate thoughts (e.g., obsessions, worries) are associated with the general dimension of anxiety across obsessive- compulsive disorder (OCD) and non-OCD anxiety disorders, suggesting that deficits of inhibitory control may contribute to anxiety symptoms across conventional diagnostic boundaries. Both OCD and non-OCD anxiety disorders often emerge as youth transition from childhood into adolescence and, theoretically, atypical development of anterior cingulate–based mechanisms for inhibitory control could underlie the onset and early course of these disorders. Testing for impaired anterior cingulate cortex (ACC) capacity for inhibitory control in pediatric OCD and non-OCD anxiety disorders represents an important first step towards establishing this neurocognitive function as relevant for the emergence of anxiety across traditional diagnostic categories.
Methods: 19 OCD, 18 non-OCD anxiety disorder (specific phobia, separation anxiety disorder, generalized anxiety disorder, social phobia) and 23 healthy female subjects (8 to 18 years) were required to resolve cognitive conflict, a measure of inhibitory control, on the Multi-source Interference Task whileforty oblique T2* weighted images were acquired on a 3T GE Magnet (TR = 2000 ms, TE = 30 ms, flip angle = 900, FOV = 20 cm, Freq = 64, 3 mm/slice). An ANOVA was used to test for differences in brain activation to conflict processing between OCD, non-OCD anxiety disorder, and healthy subjects while covarying age in SPM5.
Results: A group difference in conflict-related activation of the ACC (12, 0, 48; Z = 3.72; k = 20) was observed. Post-hoc t-tests were conducted using contrast estimates extracted from this area, revealing greater activation in OCD ($p < .001$) and non-OCD anxiety disorder patients ($p = .006$) compared to healthy controls, but no difference between patient groups ($p = .17$). Outside of the ACC, whole brain analysis revealed an area of difference in the left putamen (-21, 0, 6; Z = 3.23; k = 19)which, again, was driven by greater activation in OCD ($p = .006$) and non-OCD anxiety disorder patients ($p = .002$) than healthy subjects but no difference between patient groups ($p = .48$).
Discussion: Exaggerated ACC response to conflict was found to generalize across OCD and non-OCD anxiety disorders in pediatric patients, suggesting that ACC-based conflict monitoring represents a common neural substrate for pediatric anxiety disorders at the early stages of illness. In addition, excessive conflict-related engagement of the left putamen in both patient groups extends theories of cortico-striatal involvement in pediatric OCD to other forms of pediatric anxiety.
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124. Structural Brain Differences in PTSD are Associated with Impaired Fear Inhibition

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Background: Our previous studies showed that PTSD patients with the highest symptoms have impaired inhibition of fear-potentiated startle in the presence of safety cues (Jovanovic et al., 2009; Norrholm *et al.*, 2010). Previous studies have found structural volumetric changes in prefrontal and insular cortex in subjects with PTSD (Chen *et al.*, 2006), as well as altered insular and anterior cingulate cortex activity in PTSD (Strigo *et al.*, 2010; Hopper *et al.*, 2007). In addition, animal research indicates that the insular cortex is involved in safety signal processing (Christianson *et al.*, 2011). The current study aimed to see whether these structural changes are present in a highly traumatized civilian population, and whether they are associated with impaired fear inhibition to safety signals.
Methods: The study sample was recruited from a highly traumatized urban population at Grady Hospital in Atlanta, GA. We recruited women with a history of trauma (n = 53), with (n = 26) and without PTSD (n = 27). The participants were assessed for PTSD symptoms using the Posttraumatic Symptom Scale (PSS). We measured fear-potentiated startle responses to danger and safety cues using EMG recordings of the *orbicularis oculi* (eyeblink) muscle prior to conducting a T1-weighted (MP-RAGE) structural magnetic resonance imaging (MRI) scan in a 3T Siemens Magnetom Trio scanner. We employed the global and local shape analysis workflows implemented in the Laboratory of Neuro Image (LONI) Pipeline, which include voxel and tensor based morphometry and generalized linear modeling, for analyzing between group anatomical differences and covariates of structural changes and subject phenotypes.
Results: Structural analyses indicated reduced volume in PTSD participants compared to trauma controls in the insular cortex ($p = 0.01$), cingulate gyrus ($p < 0.02$), and caudate nucleus ($p < 0.05$). Furthermore, the volume of the insular cortex was inversely correlated with fear-potentiated startle in the presence of safety cues ($p = 0.008$), suggesting that a smaller insular cortex is associated with decreased inhibition of fear.
Discussion: These preliminary results suggest that PTSD-related reductions in volume of brain structures that have been reported in the literature were replicated in our highly traumatized civilian sample. These structural changes appear to be associated with psychophysiological markers of dysregulated fear responses in PTSD. The insular cortex may be one of the key neuroanatomical structures involved in impaired fear inhibition.
Disclosure: D. Gutman: None. **K. Ressler:** Part 1: Dr. Ressler has received awards and/or funding support within the last two years from Burroughs Wellcome Foundation, NARSAD, NIMH, NIDA. He is also a founding member of Extinction Pharmaceuticals and Therapade Technologies, which have provided no equity or income, but in which he is a stakeholder.. **N. Fani:** Part 1: Dr. Fani has funding from NIMH. **T. Ely:** None. **E. Glover:** None. **S. Norrholm:** Part 1: Dr. Norrholm has funding from NARSAD and DoD. **O. Kiefer:** None. **I. Dinov:** Part 1: Dr. Dinov's laboratory receives research funding from NIH and NSF. **A. Zamanyan:** None. **A. Toga:** Part 1: Dr. Toga has funding from NIH and NSF grants. **T. Jovanovic:** Part 1: Dr. Jovanovic receives research funding from NIMH and NARSAD.

125. Neural and Behavioral Correlates of Peritraumatic Dissociation in an Acutely Traumatized Sample

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Background: Peritraumatic dissociation has been identified as a strong predictor of subsequent posttraumatic stress disorder development. Wetherefore aimed to examine the mechanism by which peritraumatic dissociation is related to PTSD development-

by exploring the neural correlates of peritraumatic dissociation during posttraumatic adjustment.
Methods: We combined a prospective questionnaire study with a neuroimaging paradigmin an acutely traumatized sample of 121 acutely traumatized subjects. Individuals were assessed for trauma and dissociative symptoms at three time points within the first three months post trauma. A subsample of 21 subjects underwent a script-driven 4T-MRI-scan two to four months post trauma.
Results: Peritraumatic dissociation predicted PTSD diagnostic status. Peritraumatic dissociation scores were positively correlated with activation in the right occipital lobe, i.e. the lingual (BA 18, $Z = 3.37$), fusiform (BA 19, $Z = 3.64$) and parahippocampal (BA 19, $Z = 3.25$) gyri. After covariation of state dissociation, peritraumatic dissociation remained positively correlated with activation in the right lingual (BA 18, $Z = 3.21$) and fusiform (BA 19, $Z = 3.55$) gyri.
Discussion: The neuroimaging findings indicate that peritraumatic dissociation is associated with greater activation of the right occipital lobe (BAs 18 and 19), a region previously implicated in vivid autobiographical memory recall of highly emotional events. These results suggest that peritraumatic dissociation directly leads to the formation of intrusive memories.Peritraumatic dissociationand childhood trauma emerged as valuable predictors of PTSD development and therefore can guide the identification of individuals at risk.
Disclosure: R. Lanius: None. **J. Daniels:** None. **N. Coupland:** None. **K. Hegadoren:** None. **B. Rowe:** None. **R. Neufeld:** None.

126. Reduced Amygdala Serotonin Transporter Binding in Posttraumatic Stress Disorder Revealed by [^{11}C]AFM and Positron Emission Tomography

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Background: The amygdala is a key site where alterations in the regulation of the serotonin transporter (5-HTT) may alter stress response. Deficient 5-HTT function and abnormal amygdala activity have been hypothesized to contribute to the pathophysiology of posttraumatic stress disorder (PTSD), but no study has evaluated the 5-HTT in humans with PTSD. Based upon translational models, we hypothesized that patients diagnosed with PTSD would exhibit reduced amygdala 5-HTT expression as measured with positron emission tomography (PET) and the recently developed 5-HTT-selective radiotracer [^{11}C]AFM.
Methods: Fifteen participants with PTSD and 15 healthy control (HC) subjects without trauma history underwent a resting-state PET scan.
Results: [^{11}C]AFM binding potential (BP_{ND}) within the combined bilateral amygdala ROI was significantly reduced in the PTSD group compared to the HC group ($p = 0.027$; 16.3% reduction), which was largely driven by the between-group difference in the left amygdala ($p = 0.008$; 20.5% reduction). Further, amygdala [^{11}C]AFM BP_{ND} was inversely correlated with both HAM-A scores ($r = -0.55$, $p = 0.035$) and MARDS scores ($r = -0.56$, $p = 0.029$).
Discussion: Our findings of abnormally reduced amygdala 5-HTT binding in PTSD and its association with higher anxiety and depression symptoms in PTSD patients support a translational neurobiological model of PTSD directly implicating dysregulated 5-HTT signaling within neural systems underlying threat detection and fear learning.

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127. fMRI Study of Fear Acquisition and Extinction in Posttraumatic Stress Disorder

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Background: Failure to attenuate learned fear response to traumatic stimuli is a central feature of posttraumatic stress disorder (PTSD). Recent studies have pointed to abnormal brain activation patterns in PTSD patients compared with trauma-exposed healthy controls (TE-HC). Impaired fear extinction recall was found to be associated with deficient functional activation of various nodes of the fear extinction network, involving the ventromedial prefrontal cortex (vmPFC), amygdala, and hippocampus (Milad*et al*, 2009; Shin*et al*, 2005). Further clarification of fear circuitry abnormalities in PTSD can facilitate identification of biomarkers associated with onset, persistence and recovery from PTSD.

Using the fear conditioning and extinction paradigm of Milad and colleagues, in this NIMH funded study (2R01MH072833), we aimed to: a) test the reproducibility of deficient activation of the fear extinction network in PTSD, b) extend those findings by examining potential differences between groups during fear renewal and contextual shifts, and c) determine the effects of Prolonged Exposure (PE), a front-line PTSD intervention, on the fear learning and extinction circuitry. We present preliminary results from this ongoing study.

Methods: PTSD patients and a TE-HC group underwent a 2-day aversive conditioning and extinction task (Milad *et al*., 2007) during fMRI in a 1.5-T scanner. Skin conductance response (SCR) was assessed throughout the experiment as an index of conditioned response (CR). During Conditioning on Day 1, subjects developed a CR to two conditioned stimuli (CS+: red and blue lights) that were paired with an aversive unconditioned stimulus (US), a mild electric shock, in the "fear" context; a third light (yellow) was also presented but never followed by shock (CS-). During Extinction training on Day 1, only one of the two CS+'s was presented without the US (extinguishing the conditioned fear of this CS+), in the "safe" context. On Day 2, Recall of the extinction memory was tested when the three lights, the extinguished CS+ (CS+E), the CS+ that was not extinguished (CS+NE) and the CS-, were presented in the "safe" context. During Renewal, the subjects were presented with the three CSs again, but within the "fear" context.

Results: The SCR data demonstrated successful differential Conditioning acquisition within each group and no between-group differences thus far. Marginal increase in SCR in the PTSD group during the end of fear Extinction training was observed. During Day 2 Recall, the TE-HC group showed lower response to the CS+E compared to the non-extinguished CS+NE, whereas the PTSD group did not, indicating impaired recall of extinction memory in PTSD subjects. During fear Renewal testing, TE-HC subjects showed similar high response between CS+E and CS+NE, suggesting intact memory of the "fear" context. Interestingly, PTSD subjects showed elevated response to CS+E, but not CS+NE. Calculation of percent extinction learning index (ELI) and extinction recall index (ERI) largely support the aforementioned SCR data (ELI: 62% vs. 85%, ERI: 40% vs. 65%, for PTSD and TE-HC respectively). Analysis of fMRI data during the four experimental phases showed the following activation patterns in PTSD patients compared to TE-HCs: during Conditioning, decreased vmPFC response to CS+ compared to CS- as well as an increased response in bilateral hippocampus; during Extinction learning, less vmPFC activation to the CS+ (compared to CS-) in PTSD patients; and during extinction Recall, decreased vmPFC and hippocampus response to the extinguished CS+ compared to the non-extinguished CS+ in PTSD relative to controls. Moreover, during Renewal, increased activity in the head and body of caudate was observed for extinguished versus non-extinguished CS+ in PTSD relative to controls. Preliminary data on the differences between pre-treatment and post-treatment data are currently being collected and will be presented.

Discussion: The data gathered thus far replicate previous findings showing the fear extinction network is impaired during extinction recall in PTSD. Importantly, our current study extends the existing data in providing novel findings regarding differences in the responsivity of the fear extinction network during fear learning and fear renewal. The findings support the hypothesis that PTSD is associated with abnormal functioning of brain circuitry underlying fear conditioning and extinction, and provide preliminary evidence for an effect of PE on fear-related brain circuitry. Theoretical and clinical implications will be discussed.

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128. Towards the Neural Basis of Attentional Training Effects in Social Anxiety

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Background: The tendency to preferentially allocate attention toward threat-relevant information is considered an important mechanism in the pathogenesis and maintenance of anxiety (e.g., Mathews & MacLeod, 2005). Recently, researchers have used a computerized cognitive training procedure to modify attention to emotional stimuli and have demonstrated that experimentally manipulating attentional allocation toward threatening information increases anxiety in response to stress (e.g., Clarke *et al*., 2008; MacLeod *et al*., 2002) whereas decreasing attentional bias for threat attenuates anxiety reactivity (See *et al*., 2009) and reduces symptoms in clinically anxious populations (e.g., Amir *et al*., 2009; Schmidt *et al*., 2009). Although these findings suggest that attentional modification procedures may reduce vulnerability toward as well as facilitate recovery from anxiety-related conditions, little is known about the underlying neurobiological mechanisms that account for such effects. As a first step toward addressing this issue, we examined the neural correlates of a well-established attentional training procedure in a sample of individuals with elevated levels of social anxiety.

Methods: Fourteen volunteers endorsing at least moderate levels of social anxiety (Liebowitz Social Anxiety Scale 40; $M = 79.07$, $SD = 22.29$) took part in a functional magnetic resonance imaging (fMRI) session in which they completed an emotional face processing task before and after an attentional modification

procedure designed to facilitate disengagement from threat-relevant cues. The emotion face assessment paradigm required participants to match one of two probe faces to a target face according to the emotional expression displayed (i.e., angry, fear, or happy). In the control condition, participants matched the orientation of target and probe ovals. This task has been shown to reliably engage limbic structures during emotional face processing (e.g., Hariri *et al*., 2002) and distinguish anxiety-prone and non-anxious individuals (Stein *et al*., 2007). To train attention away from threat, participants completed a modified probe detection task in which they identified a visual probe that consistently appeared following neutral stimuli during the presentation of threat-neutral stimuli pairs (Amir *et al*., 2008). Following the MRI scan, participants were asked to deliver a 5-minute videotaped speech. State anxiety was measured before and after the MRI scan as well as following the speech task. The fMRI analysis focused on examining changes from before to after training in the contrast (% signal difference) between processing of emotional faces and shapes. A linear mixed model analysis was computed using R (www.cran.org). We also examined whether changes in neural activation on the face processing task from before to after training predicted response to the behavioral stressor (i.e., anxiety reactivity).

Results: Participants displayed a significant reduction in activation from pre- to post-training in the bilateral amygdala and insula as well as significantly increased activation in the left ventral medial prefrontal cortex (vmPFC) in response to emotional faces. These neural changes occurred in the absence of changes in participants' level of self-reported state anxiety from before to after training. Critically, greater attenuation in amygdala activation as well as increased activation in the left vmPFC following training predicted less anxiety reactivity to the speech challenge.

Discussion: Attentional training altered activity in brain regions that are important for modulating the processing of emotional information and that have been implicated in the pathophysiology of anxiety (e.g., Etkin & Wager, 2007). Specifically, decreased activation in the amygdala and insula along with increased activation in the vmPFC may reflect improvements in top-down regulation of emotion. Limitations of the present study include the use of a non-clinical sample, lack of an experimental control group, and small sample size. The current findings support the notion that integrating attentional training procedures with fMRI can provide important information about the neural mechanisms that regulate the processing of emotional information and that may confer heightened vulnerability to anxiety.

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129. Cognitive Behavioral Therapy In Obsessive-Compulsive Disorder Changes Connectivity Between Anterior Insula and Default Mode Network

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Background: Obsessive-compulsive disorder (OCD) is characterized by an excessive focus on negative internally-generated thoughts, feelings, or images. Internally-focused thought processes are subserved by the "default mode network" (DMN), which has been found to be hyperactive in OCD during cognitive tasks. In healthy individuals, disengagement from internal focus may rely on competition for behavioral control between DMN and a fronto-parietal network (FPN) associated with external attention and task execution. Using resting-state functional connectivity (rs-fcMRI) analyses, we have previously identified impairment in competitive interactions (negative correlations) between the anterior insula, a key node of FPN, and default mode regions in OCD; the current study investigated rs-fcMRI with anterior insula before and after cognitive behavioral therapy (CBT) using exposure and response prevention (ERP).

Methods: Two sessions of rs-fcMRI data were obtained from 9 unmedicated patients with OCD and 7 healthy control subjects. Sessions were separated by approximately twelve weeks, during which time patients received CBT using ERP from two highly experienced clinicians. Timecourses from bilateral seeds in anterior insula were correlated across the whole brain for both sessions. Regions showing group differences in connectivity with anterior insula seeds before CBT (corrected for multiple comparisons at $p < .05$) were submitted to two-way ANOVAs testing for interactions between group and time (before vs. after CBT).

Results: Symptom severity as measured by the Yale-Brown Obsessive Compulsive Scale (YBOCS) decreased in all patients after twelve weeks of CBT, with the group as a whole exhibiting a significant reduction after CBT (mean before vs. after: 25.9 vs. 16.8, $p<.001$). Before CBT, OCD patients showed less negative connectivity than controls between left anterior insula (-35, 14, 6) and several regions of DMN, including dorsomedial prefrontal cortex (15, 39, 51, $z = 4.1$) posterior cingulate cortex/precuneus (-15, -84, 30, $z = 3.7$; 21, -54, 12, $z = 4.0$; -24 -87, -9, $z = 4.3$), and posterior inferior parietal cortex (48, -81, 27 $z = 3.3$; 48, -69, 6, $z = 4.0$). Therapy normalized connectivity between these regions in patients, such that group differences found prior to CBT were reduced or abolished following CBT.

Discussion: OCD patients exhibit abnormal connectivity between anterior insula and DMN during rest, which may contribute to patients' inability to disengage from default mode-based internally generated scenarios and thoughts in order to focus attention on external tasks. Although the small sample size from this pilot study necessitates cautious conclusions, changes in resting-state functional connectivity between anterior insula and DMN with CBT may point to a mechanism of treatment.

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130. Increased Risk for Affective Disorders Programmed In Utero? High Prenatal Maternal Cortisol and Size of the Amygdala in the 6-9 Year-old Offspring

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Background: Because fetal brain development proceeds at an extremely rapid pace, early life experiences have the potential to alter the trajectory of neurodevelopment. Alterations especially in limbic structures have been associated with a range of neuropsychiatric disorders, including affective disorders. Studies in non-human primates and rodents have shown that such alterations can be induced in the offspring of mothers by prenatal exposure to exogenous glucocorticoids or chronic stress. To this date, the association between exposure to prenatal maternal cortisol concentrations and size of limbic structures has not been studied in human subjects.

Methods: In the current prospective longitudinal study we included women for whom serial data on cortisol concentrations were available at five time points over the course of gestation. When the offspring from the target pregnancy were between six to nine years of age, volumes of the amygdala were assessed by manual segmentation of T1 magnetic resonance (MR) images, acquired by a Phillips 3T Tesla.

Results: After controlling for potentially confounding postnatal factors, high maternal cortisol concentrations were associated with larger amygdala volumes in the 6-9 year old offspring. Furthermore, high maternal prenatal cortisol concentrations were associated with more affective problems in their offspring and this association was mediated by larger amygdala volumes. Analyses stratified by sex suggested that these associations were significant in female but not in male offspring.
Discussion: These findings are in line with studies in rodents and non-human primates and suggest that higher maternal cortisol concentrations during pregnancy are associated with changes in limbic structures, which may increase the offspring's susceptibility for neuropsychiatric disorders.
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131. Dynamic Causal Modeling of Incentive Anticipation in Healthy Adults and Adolescents

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Background: Animal and human studies suggest that the reward system may drive the increase in risk-taking behavior typical of adolescence, though the neural mechanisms remain unclear. While previous studies have focused on developmental differences of individual brain regions, few studies have examined connectivity differences during incentive processing. Regions consistently engaged by incentive processing include the nucleus accumbens, thalamus, and insula. To further understand how these regions interact as a network during incentive processing, functional magnetic resonance imaging (fmri) and dynamic causal modeling (DCM) were used to examine effective connectivity among these three nodes in typical adults and adolescents who performed the well-accepted monetary incentive delay (MID) task (Knutson *et al.*, 2001).
Methods: 24 typical adolescents and 30 typical adults performed the event-related MID task in which $0.20, $1 or $5 could be won or lost if they responded fast enough to a visual target. Fmri preprocessing, general linear model (GLM) and connectivity analyses of the cue-elicited anticipatory phase were conducted with SPM8. Nucleus accumbens, anterior insula, and medial (including midline and mediodorsal) thalamus were chosen *a priori* to model an incentive network, based on their strong activation in this study and other MID studies in typical subjects. Six connectivity models were estimated for gain and loss anticipation and underwent Bayesian Model Selection (BMS) to determine the best-fit model. All models were constrained to known anatomic connections delineated by tract-tracing studies in non-human primates. Connection strengths were extracted from the best-fit model, examined for significance, and entered into a linear mixed model with group (adolescent, adult), valence (gain, loss) and connection (between regions) as factors.
Results: As expected, GLM analysis revealed activation of ventral and dorsal striatum, thalamus, insula, and globus pallidus ($p<0.005$, uncorrected, >10 voxels) for contrasts of all gain or all loss cues versus the neutral cue in each group, indicating expected task effects. Following BMS, two equally best-fitting models emerged, and were the same for each group. These models differed only by the presence or absence of a single connection, and the more complete model was further explored. In the adult group, all gain cues significantly modulated the thalamo-accumbens connection, while all loss cues modulated the thalamo-accumbens, thalamo-insula, and insula-accumbens connections. In adolescents, all gain cues significantly modulated the thalamo-insula connection, while all loss cues modulated the thalamo-accumbens, insula-thalamus, insula-accumbens, and accumbens-thalamus connections. No statistically significant between-group differences were found.
Discussion: This study gives evidence for direction-specific interactions between nucleus accumbens, thalamus and anterior insula during cue-elicited incentive anticipation. In both groups, thalamic and/or insula activity influenced accumbens activity during gain or loss cue-elicited anticipation, suggesting novel functional influences on the 'limbic-motor interface'. Furthermore, adults and adolescents appeared to recruit this network in a similar manner during incentive processing. Future work may include applying this network to other phases of reward processing, or expanding the modeled neural network.
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132. Reorganization of Somatosensory Cortex in Adult Women with Histories of Childhood Abuse

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Background: Early experience has profound organizing effects on the developing brain. While experience-guided plasticity is critical for normal brain development, the developing brain is also particularly sensitive to the untoward effects of adverse experiences which may permanently alter the organization of the brain, leading to long-lasting detrimental behavioral outcomes. We here studied the effects of exposure to childhood abuse on cortical thickness in adult women.
Methods: We recruited 56 medically healthy adult women aged 18-45 years with/without histories of childhood abuse and/or current major depression. Histories of childhood abuse were quantified using the Childhood Trauma Questionnaire (CTQ). For the present analysis, CTQ cutoff scores for moderate-severe exposure were used to classify subjects into those with versus those without a history of abuse prior to age of 13 years. High-resolution T1 weighted anatomical MRI images were collected, non-uniformity corrected, and registered into stereotaxic space using a neural net classifier. After tissue classification, whole-brain white and gray matter surfaces were then fitted using deformable model algorithms. Cortical thickness is defined as the distance between linked vertices of white and gray matter surfaces. Statistical analysis was performed at every vertex, regressing cortical thickness of the exposure group versus controls, while controlling for age and depression. The resulting statistical maps were corrected for multiple comparisons using the false discovery rate (FDR) at a q value of 0.05.
Results: Regression analyses revealed that exposure to early childhood abuse was explicitly associated with altered primary somatosensory cortex thickness in adult women. Specifically, the representation of the genital area in the somatosensory cortex (BA 3, cluster centered at Talairach coordinates xyz 2 -37 70) was significantly decreased in victims of childhood abuse compared to non-abused controls ($q<0.05$). Further analyses explore whether decreased somatosensory representation of the genital area is specific to sexual abuse exposure and associates with earlier onset age of the abuse as opposed to cumulative exposure.
Discussion: Our resultsprovide the first evidence that childhood abuse induces long-lasting reorganization of the primary somatosensory cortex with altered genital representation, supporting the concept of experience-dependent cortical plasticity in humans.

Future studies should explore whether this somatosensory reorganization after early abuse translates into clinical symptoms, such as sexual dysfunction.

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133. Social Subordination Stress and Serotonin Transporter (5HTT) Polymorphisms Affect the Development of Brain White Matter Tracts in Juvenile Female Macaques: Behavioral and Neuroendocrine Correlations

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Background: Social subordination is a potent social stressor in rhesus monkeys, associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and adverse health outcomes in adult females. Social subordination also delays puberty and increases emotional reactivity, and all these effects are exacerbated by the short (s) 5HTT allelic variant. This study investigated how social subordination stress interacts with 5HTT polymorphisms to affect brain development. We focused on the developmental impact on brain white matter tracts and its behavioral and neuroendocrine correlates during the juvenile (prepubertal) period.

Methods: Diffusion tensor imaging (DTI) scans were collected on 35 socially-housed juvenile rhesus monkey females at prepuberty (19-23 mos). Based on the outcome of aggressive encounters, 13 subjects were considered dominant and 22 subordinate. Half the animals in each rank category had both long promoter length alleles (l/l) of the gene encoding the 5HTT and half had at least one short allele (s-variant). DTI data (single-shot double spin-echo EPI sequence, GRAPPA (R = 3), voxel size: 1.3x1.3x1.3 mm3, TR/TE = 5000/86 ms, FOV: 83 mm, b:0, 1000 s/mm^2, 60 directions, 12 averages) was analyzed using tract-based spatial statistics (TBSS; FSL, Oxford), which generated skeletonized, co-registered fractional anisotropy (FA) maps of the major white matter tracts for all subjects, then analyzed using a voxelwise Two Way ANOVA for rank and 5HTT genotype. Tracts were identified using the tractography functions of Slicer software (v. 3). In order to assess the functional implications of the brain structural alterations, behavioral measures of emotional reactivity (collected in response to the Approach/Avoidance and Human Intruder tasks) and neuroendocrine measures of stress reactivity (cortisol elevations in response to an acute, 30 min, separation stress paradigm) were correlated with DTI FA measures.

Results: Three clusters of voxels showed rank-dependent differences in medial prefrontal cortical white matter (local medial prefrontal fibers, some of them cross-hemispheric), and in the white matter along the dorsal medial wall (mainly cortico-thalamic tracts connecting somatosensory and parietal cortices with thalamic regions). Low ranking animals had higher FA in all these regions as compared to high ranking animals. Main effects of 5HTT polymorphisms were detected in an extensive cluster of voxels in the left posterior limb of the internal capsule that could include corticorubral tracts, where l/l animals had lower FA than s-variants. Rank by 5HTT interaction effects were found in other three clusters, one possibly corresponding to the fronto-occipital fasciculus, a second in the right parietal white matter (short range "U" fibers) and the third in the left prefrontal white matter (short range fibers connecting medial and dorsolateral PFC). In all three of these clusters high ranking l/l animals had lower FA than s-variant ones, and l/l low ranking animals had higher FA than s-variant ones. FA in the regions where we found significant rank and/or 5HTT genotype effects was correlated with submissive, fearful and anxious behaviors, indicating that these differences have functional relevance. Although FA in these regions did not correlate with HPA axis stress reactivity measures, voxelwise correlation analysis did find significant correlations between FA in parietal white matter and cortisol stress response.

Discussion: The differences in FA detected by DTI reflect social stress-induced alterations in the development of brain white matter tracts connecting prefrontal, association and sensory processing brain regions, sometimes modulated by 5HTT allelic variants. These altered tracts could affect emotional and sensory processing. Most of these alterations were correlated with increases in emotional, but not HPA axis, reactivity. These findings highlight the important developmental impact of social stress on brain structural connectivity and its emotional correlates.

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134. Healthy Pediatric White Matter Development: A Diffusion Tensor Imaging Study and Meta-Analysis

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Background: There is increasing evidence that white matter abnormalities play a role in psychiatric disorders that typically develop in adolescence. Studying healthy human neurodevelopment may provide insight into the aberrant processes involved in the development of psychiatric disorders. In this study we thus examined healthy age-associated changes in white matter tracts using diffusion tensor imaging (DTI).

Methods: DTI was performed in 78 healthy subjects, 8-21 years old (mean age 15.3 years, SD = 3.7), at 3T with a slice thickness of 2.5 mm, and 31 non-collinear directions (b = 1000 s/mm^2) and 5 volumes without diffusion weighting. We used tract-based spatial statistics (TBSS) to examine the relationship between age and fractional anisotropy (FA), a putative measure of white matter integrity. In addition, we performed a meta-analysis of five other DTI-TBSS studies that examined white matter development in healthy individuals (n = 239; mean age 15.5-17.5 years; age range = 8-32). Three studies analyzed age-FA correlations using within-group correlations and two studies compared children with young-adults. One study was longitudinal and four were cross-sectional. The peak-coordinates of significant FA changes were meta-analyzed using the signed differential mapping (SDM) software.

Results: In our cohort we identified bilateral clusters where age correlated positively with FA, including the bilateral superior longitudinal fasciculus and the left limb of the internal capsule, which survived strict family-wise error correction ($p < 0.05$). The independent meta-analysis was consistent with these results and revealed significant positive associations of age with the bilateral superior longitudinal fasciculus, the left limb of the internal capsule, and the right dorsal cingulate ($p < 0.001$; p-values were obtained from a randomization test).
Discussion: DTI studies indicate increases in white matter integrity in adolescence, possibly reflecting increased myelination and/or an increase in axonal diameter. The bilateral superior longitudinal fasciculus (SLF) and the left limb of the internal capsule in particular appear to undergo active development in adolescence. The SLF has been implicated in language functions, and connects Broca's and Wernicke's areas. In this regard it is particularly noteworthy that SLF abnormalities have been linked to auditory hallucinations in schizophrenia. The left limb of the internal capsule mainly contains fibers of the thalamic radiation, which has been reported to be compromised in schizophrenia, bipolar disorder and other psychiatric disorders. Thus, abnormal microstructural development of the SLF and thalamic radiation may be implicated in the pathophysiology of schizophrenia and other psychiatric disorders.
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135. Amphetamine Effects on MATRICS Performance in Healthy Adults: Prelude to a Genetic Analysis

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Background: The ability to predict drug effects on neurocognition in individuals will become increasingly important, as we move to biomarker-strategies for pro-cognitive therapies. We study predictors of neurocognitive sensitivity to drug challenges in healthy control subjects (HCS). En route to the requisite sample size for genetic analyses of amphetamine (AMPH) sensitivity, we now report interim findings of AMPH effects on HCS performance on the MATRICS Consensus Cognitive Battery (MCCB).
Methods: To date, 45 HCS (M:F = 23:22; age (y) mean (range) = 23.8 (18-35)) have completed a double blind, cross-over, placebo (PBO) controlled study of AMPH (20 mg) effects on MCCB performance. HCS completed 2 test days (430 min/day), separated by 1 month. 110 min post-pill, subjects completed the MCCB, which measures performance in domains of speed of processing (SP), attention/vigilance (A/V), working memory (WM), verbal learning (VL), visual learning (VIS), reasoning and problem solving (RPS) and social cognition (SC). Autonomic measures and self-rating scales were monitored. Blood was stored for genetic analyses.
Results: Autonomic and self-rating scores supported AMPH bioactivity. Independent of drug, significant MCCB order effects (Test 2 > 1) were noted for SP, A/V, WM, VL and VIS (all p's < 0.004-0.0001). AMPH tended to impair WM but otherwise had no significant main effect on any individual MCCB domain T-score. After controlling for order effects, the "AMPH effect" (AMPH score - PBO score) was inversely related to performance on PBO day for SP ($p < 0.05$), A/V ($p = 0.001$), WM ($p = 0.003$), VL ($p < 0.001$), RPS ($p = 0.002$) and SC ($p < 0.03$). AMPH effects on self-rated drowsiness correlated significantly with performance in SP, A/V and WM domains.
Discussion: Conclusion: Interim findings support inter-individual differences in AMPH effects on MCCB performance based on: 1) basal domain performance, and in some cases, 2) AMPH effect on drowsiness. The relationships between AMPH impact and basal MCCB performance levels persist when controlling for the potential impact of order effects. These findings are consistent with other reports of AMPH's "inverted-U" impact on frontal lobe function - increasing low basal levels of frontal activation and reducing higher basal levels - and set the stage for planned analyses of the genetic determinants of AMPH neurocognitive sensitivity.
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Disclosure: N. Swerdlow: Part 1: Neurocrine, Inc. - Consultant. **J. Talledo:** None. **J. Kei:** None. **S. Thanam:** Part 1: Neurocrine, Inc. - Consultant. **S. Lamb:** None. **G. Light:** None.

136. Does Birthweight directly impact Later Neurocognitive Outcomes? A Strict Test using Longitudinal Neuroimaging in Monozygotic Twins

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Background: In humans, lower birth weight (BW) has been repeatedly linked to adverse cognitive and behavioral outcomes, and more recently via *in-vivo* neuroimaging, to altered postnatal neuroanatomy. However, few BW studies have been able to adequately control for confounds such as gestational age, parental age, race and family socioeconomic status. Furthermore, the lack of longitudinal neuroimaging studies in this field means that little is known regardlng the relationship between BW variation and patterns of structural brain development in postnatal life.
Methods: To carry out a strict test of the hypothesis that BW variation is directly relevant for later neurodevelopment, we related BW differences (BWd) within 80 monozygotic (MZ) twin-pairs to within-pair differences in cognitive ability, and longitudinal measures of brain anatomy gathered between ages 5 and 22 years. This approach simultaneously controls for multiple genetic and environmental confounds that might otherwise lead to spurious over- or under-estimation of associations between BW and later outcomes. We hypothesized that BW reductions in lighter twins (LTs) relative to heavier twins (HTs) would (i) negatively impact cognitive ability, (ii) exert developmentally dynamic and sexually dimorphic influences on post-natal brain maturation, and (iii) most alter aspects of brain anatomy - like total brain volume (TBV) and cortical surface area (SA) - with established sensitivity to prenatal insults. We also investigated the relationship between BWd and differences within twin pairs for measures of psychopathology.
Results: Differences in BW were quantified as [(LT weight – HT weight)/HT weight]. Within-pair neuroanatomical differences were similarly quantified from 192 pairs of longitudinally acquired structural magnetic resonance imaging brain scans. Mixed-models were used to statistically assess anatomical differences between LTs and HTs, and how these differences varied by age, extent of BWd and sex. Full Scale and Performance IQ were significantly lowered in LTs. Cognitive impairments were accompanied by (but not statistically associated with) systematic LT-HT differences in brain development. Total brain, white matter (WMV), gray matter (GMV) and cortical volume (CV) were all significantly reduced in LTs compared to HTs and this effect was strongest for TBV. These deficits grew with increasing BWd, and this "dosage effect" was developmentally stable for GMV and CV, but emerged over adolescence for TBV and WMV. The impact of BWd on CV was entirely mediated by SA, rather than cortical thickness - reflecting complex and distinct consequences of BWd for SA's two determinants - total cortical hull surface area (determined by radial brain size) and the degree of cortical gyrification. We will

also present data regarding the relationship between BW and later behavior.
Discussion: Our data argue that in healthy full-term pregnancies, BW variation captures aspects of the in-utero environment with strong and developmentally dynamic consequences for neurodevelopment throughout childhood and adolescence.
Disclosure: A. Raznahan: None. **L. Clasen:** None. **F. Lalonde:** None. **J. Blumenthal:** None. **J. Giedd:** None.

137. Effects of Early Life Stress on Infant Macaque Brain Development: A Longitudinal Study of Structural and Functional Connectivity Changes using Diffusion Tensor Imaging and Resting State fMRI.

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Background: Early adverse experiences, especially those involving mother-infant relationship disruption, are detrimental for proper socioemotional development in primates. Humans with histories of childhood maltreatment, such as abuse and neglect, are at higher risk for developing psychopathologies including depression, anxiety, substance abuse and other behavioral disorders. The underlying neurodevelopmental alterations are not well understood due to limitations of research with maltreated human populations, but are thought to involve brain circuits that control emotional regulation and processing including the ventro-medial prefrontal cortex (vmPFC) and the amygdala. Here we studied the effects of infant maltreatment in nonhuman primates as a model organism to investigate the complex relationship between early caregiving experiences and infant brain development. The goals of this longitudinal study were to investigate the impacts of this early adverse experience on the development of neural networks involved in emotional regulation in rhesus monkeys and to examine how they unfold during infancy in relationship to behavioral alterations. For this, we used *in vivo* neuroimaging approaches, specifically diffusion tensor imaging (DTI) and resting state functional connectivity MRI (rs-fcMRI).
Methods: Thirty-six rhesus monkey infants were cross-fostered at birth and randomly assigned to either maltreating mothers (who exhibited both physical abuse towards and intense rejections of their very young infants; Malt, $n = 18$) or to control mothers (C, $n = 18$). These 36 mother-infant rhesus pairs lived in social groups, where their behavior, neuroendocrine function and physical growth was intensely characterized. DTI scans were collected on all the infants at 2 weeks, 3 and 6 months of age. DTI allows investigations of the structural integrity of white matter tracts, such as those involved in emotion control. DTI data (single-shot double spin-echo EPI sequence, GRAPPA ($R = 3$), voxel size: 1.3x1.3x1.3 mm3, TR/TE = 5000/86 ms, FOV: 83 mm, b:0, 1000 s/mm^2, 60 directions, 10 averages) was analyzed using bothvoxel-wise tract-based spatial statistics (TBSS; FSL, Oxford), and atlas-based tractography.
A subset of infants ($n = 6$ C, $n = 6$ Malt) -and mothers ($n = 5$ C, $n = 6$ Matl)- is undergoing additional resting state functional MRI (rs fMRI) scans at 3 and 6 (only infants) months postpartum for analysis of functional connectivity in parallel to structural connectivity. Images are acquired using an EPI sequence sensitive to BOLD contrast (TR = 3 s, TE = 30 ms, zero gap, voxel size = 1.5 mm isotropic, matrix = 64×64, FOV = 9.6 cm $\times$ 9.6 cm, 2x15 min scans) under anesthesia (standardized at 1% isofluorane, inhalation). Although analysis of rs-fcMRI is still ongoing, we examined large-scale systems organization in a subset of mothers, with 88 regions of interest chosen for analysis from prior work (Lewis, Van Essen *et al.*,, 2000) based on architectonic subdivisions in the macaque monkey, created using the caret software (Van Essen). The resting state BOLD time series are then correlated region by region for each subject across the resting state time series to create correlation matrices (Fair *et al.*, 2007, 2008, 2009) derived from those 88 *a priori* defined ROIs, including vmPFC and amygdala. For statistical comparisons, r-values within matrices are normalized and group comparisons done using t-tests.
Results: Maltreated infants had reduced structural integrity (measured as fractional anisotropy –FA-) in prefrontal-limbic tracts including the uncinate fasciculus, main white matter tract connecting vmPFC and amygdala and, therefore, involved in emotional responses. The alterations in these limbic circuits emerge between 3 and 6 months of age, when the maltreated infants exhibit higher emotional reactivity than controls during behavioral tasks. We are currently analyzing the rs-fcMRI scans to examine the effects of the structural tract changes on the functional connectivity of these limbic networks. Preliminary data obtained from the mothers rs-fcMRI identified several circuits and unique patterns of atypical connectivity between abusive and non-abusive animals, with parietal connections and connections amongst the medial and lateral temporal lobe most atypically connected.
Discussion: The structural alterations in prefrontal-amygdala tracts of maltreated infants could underlie their higher emotional reactivity in comparison to controls. Further analyses will shed light on this potential relationship, the neurobiological mechanisms involved and time course of events associated with this adverse early experience.
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138. Impact of In-Scanner Head Motion on Multiple Measures of Functional Connectivity: Relevance for Studies of Neurodevelopment in Youth

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Background: It has recently been reported (Van Dijk *et al.*, 2011) that in-scanner head motion influences MRI measurements of resting-state functional connectivity. This finding may be of particular relevance for studies of neurodevelopment in youth, confounding analyses to the extent that motion and subject age are related.
Methods: Here we expand on the findings of Van Dijk *et al.*, by examining the effect of connectivity on multiple types of resting-state connectivity analyses in a large sample of children and adolescents ($n = 458$). Following replication of the effect of motion on seed-based analyses, we examine the effect of motion on graphical measures of network topology, dual-regression of independent component analysis networks, and the fractional amplitude of low frequency fluctuation.
Results: In the entire sample, subject age was highly related to motion. Using an age and motion matched subsample, motion had marked effects on connectivity in every analysis examined. While subject age was associated with increased within-network connectivity even when motion was accounted for, controlling for motion substantially attenuated the strength of this relationship.
Discussion: The results demonstrate the pervasive influence of motion on multiple types functional connectivity analysis, and underline the importance of controlling for motion in studies of neurodevelopment. All studies that relate functional connectivity to between-group or individual differences should report and account for motion.

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139. Effect of BDNF Val66Met and Childhood Trauma on Brain Volume, Resting State Functional Connectivity and Alexithymia in Healthy Volunteers

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Background: BDNF levels are reduced both in those exposed to stress and in those who carry the Met allele of the common Val66Met polymorphism. BDNF levels affect brain development. This polymorphism has been associated with increased vulnerability to the development of psychiatric symptoms after childhood trauma and with smaller regional brain volumes in those psychiatric patients exposed to a variety of environmental stressors. Volumetric differences in brain regions likely affect functioning by altering networks in which the affected regions participate. As such, we examined the effect of BDNF Val66Met and childhood trauma on regional brain volume and used these ROIs as seeds for a whole brain connectivity analysis.
Methods: 119 (60Female) participants underwent the following assessments: Childhood Trauma Questionnaire (CTQ)- a retrospective self-report measure yielding a total score and five subscales (emotional, physical and sexual abuse and emotional and physical neglect) each ranging from "no trauma" to "extreme trauma", the Toronto Alexithymia Scale (TAS-20)- a self-report measure yielding a total score and three subscales (Difficulty Describing Feelings (DDF), Difficulty Identifying Feelings (DIF) and Externally Oriented Thinking (EOT)), Beck Depression Inventory, Wechsler Abbreviated Scale of Intelligence vocabulary subscale and Val66Met polymorphism (rs6265) genotyping using the Illumina Golden Gate platform. Participants were classified as "High CTQ" if they reported moderate to severe trauma on one or more of the five subscales. Participants were grouped by genotype as Val/Val homozygotes or Met carriers. Participants underwent whole brain anatomical scans on a 3T Siemens Allegra. A subset ($n = 78$) underwent a 6 minute resting-state EPI scan with $TR = 2$ s (rsFC).
FreeSurfer was used for cortical reconstruction and volumetric segmentation. Regional brain volume analysis was carried out using the FreeSurfer group analysis application, Qdec, at 10 mm FWHM and corrected significance of $p < 0.05$. Regions exhibiting a BDNF X CTQ interaction on volume were used as seeds for a resting state functional connectivity analysis. Time courses were extracted and averaged across each seed, then correlated with voxels across the brain. A 2 (BDNF genotype) X 2 (High/Low CTQ) ANOVA was performed with a corected p value < 0.05.
Results: In the anatomical dataset, there were 68 Val/low CTQ, 19 Met/low CTQ, 24 Val/high CTQ and 8 Met/high CTQ participants. Met allele carriers with high CTQ exhibited reduced cortical volume in left superior temporal gyrus and right inferior frontal gyrus (IFG, pars orbitalis). Using these as seeds for rsFC, we examined 48 Val/low CTQ, 8 Met/low CTQ, 18 Val/high CTQ and 4 Met/high CTQ participants. Met allele carriers with high CTQ showed significantly reduced rsFC from the inferior frontal gyrus seed to two neighboring regions of right inferior frontal cortex. Further, Met allele carriers in the whole group with high CTQ had higher scores on the TAS-20 EOT subscale. No network interactions were found for the superior temporal gyrus seed.
Discussion: Met allele carriers were uniquely sensitive to childhood trauma as exhibited by changes in cortical volume in superior temporal gyrus and IFG, changes in rsFC within right prefrontal cortex and elevated externally oriented thinking. Right IFG has been associated with a range of emotional functions including decoding emotional prosody, embarrassment and empathy. As stress and BDNF Met allele carrier status both result in reduced BDNF levels, it appears likely that these two factors may act in an additive fashion to reduce BDNF and impact cortical development and networks subserving emotional functioning resulting in altered emotional functioning after exposure to childhood stress. Our TAS-20 result indicating an elevation in EOT in Met carriers only if subjected to childhood trauma supports the literature indicating that the EOT subscale of the TAS-20 is the least heritable of the TAS subscales.
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140. Brain Network Properties in Autism, ADHD, and Typically Developing Children: Similarities and Differences

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Background: Motivated by the high prevalence of inattention and hyperactivity in individuals with autism spectrum disorders (ASD), clinicians and researchers are urging a change of the current diagnostic criterion preventing the diagnosis of Attention-Deficit/Hyperactivity Disorder (ADHD) in the presence of ASD. Yet, beyond supportive clinical data, investigations of commonalities and distinctions in the neurobiological correlates of ADHD and ASD remain scarce. This is particularly true for brain imaging studies. Specifically, there are no functional MRI (fMRI) studies directly comparing children with ASD to children with ADHD and typically developing controls (TDC). However, their separate literatures have recently revealed abnormalities in functional connectivity for both Autism and ADHD. Here, we examine distinct and shared loci of dysconnection in ASD and ADHD by means of voxel-wise network centrality, a graph-based measure of network organization in resting state fMRI data.
Methods: We compared 46 children with ASD to age- and sex-matched children with ADHD ($n = 39$) and TDC ($n = 42$). We computed two measures of voxel-wise network centrality, an index of the functional relationship of a given voxel with the entire brain connectivity matrix. We used two measures of centrality: degree centrality (DC), a local measure of the connectome graph indexing the number of direct connections for a given voxel; and eigenvector centrality (EC), a global recursive measure that depends on the degree of adjacent nodes and their own EC. We measured voxel-wise DC and EC and performed group analyses using one-way ANOVA, with group (ADHD, ASD, TDC) as the between factor, using cluster-based Gaussian random field correction for multiple comparisons ($Z > 2.3$, $p < 0.05$, corrected). To determine whether there were distinct and/or shared abnormalities between groups, we computed post-hoc pair-wise comparisons. To examine the impact of ADHD-like comorbidity on network centrality in children with ASD, we also conducted secondary group analyses including the 22 children with ADHD-like diagnosis (ASD^+) and the 23 without ADHD-like comorbidity (ASD^-) and compared these two groups to the children with ADHD and the TDC in terms of DC and EC.
Results: We found group-specific results for DC and EC in cortical and subcortical regions. Pair-wise group comparisons also revealed both shared and distinctive abnormalities in the ADHD

and ASD groups relative to TDC. For example, a significant effect of group was evident in the right putamen, caudate and thalamus. Here, secondary pair-wise comparisons of the three groups revealed significantly increased DC in the ADHD group (ASD = TDC < ADHD). By contrast, increased DC for both ADHD and ASD was observed in a cluster extending from the brainstem to the left striatum, pallidum and left mid-insula (ASD = ADHD > TDC). A significant effect of group for both DC and EC was evident in right amygdala with pair-wise post-hoc statistic showing ASD-specific increases (ASD > TDC = ADHD). Similarly, relative to TDC, both ADHD and ASD shared abnormalities in EC of the precuneus (TDC > ASD = ADHD) and of a cluster extending from the brainstem to the mid-insula (ASD = TDC > TDC). Secondary analyses comparing the children with ADHD, the TDC and the two groups of ASD with and without ADHD (ASD^+, ASD^-) revealed 1) ASD-specific abnormalities regardless of the presence of ADHD-like comorbidity, i.e., increases of both DC and EC in the right temporal pole/amygdala; 2) a centrality pattern shared by ADHD and ASD^+ but not ASD^- (e.g., increased DC in the right thalamus/striatum), 3) a centrality pattern shared by the three clinical subgroups (ASD+, ASD- and ADHD) relative to TDC, i.e., decreased DC of the posterior cingulate, and 4) angular gyrus EC increases only in the ADHD group.

Discussion: Examining whole-brain functional connectivity indexed by network centrality in children with ASD, relative to ADHD and TDC revealed abnormalities in cortical and subcortical areas previously found to be functionally disconnected in separate studies of ASD and ADHD. Direct comparisons of these groups allowed shared and distinct network properties of the disorders to be discerned. These results support clinical observations of overlap between these disorders and provide leads to for neurobiologically dissecting these highly heterogeneous conditions.

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141. Preliminary Findings from A Developmental Meta-Analysis of Neural Correlates of Autism Spectrum Disorders

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Background: Autism spectrum disorders (ASD), including both autistic disorder and Asperger's disorder, are among the most common and impairing psychiatric conditions affecting children and adolescents today. In fact, the Centers for Disease Control recently showed that the incidence of ASD had risen 10-fold from the year 1980 to the year 2000, now affecting as many as 1/150 children in the U.S. Functional magnetic resonance imaging (fMRI) studies have begun to elucidate the neural underpinnings of ASD. However, such studies are often limited by small sample size and also by a focus on either child (under 18 year old) or adult (18 years or greater) participants. To address both, we conducted an activation likelihood estimation (ALE) meta-analysis of ASD fMRI studies. ALE provides a quantitative meta-analytic technique to provide an objective statistically-based approach to examine findings across studies. Moreover, this is a novel use of ALE, as the software has only recently been enhanced to evaluate across comparisons—i.e., to compare areas of greater activation in ASD adult vs. typically-developing control (TDC) participants vs. ASD child vs. TDC. Thus, our ALE meta-analysis is three-fold: child only studies, adult only studies, and child vs. adults studies for a developmental perspective. We hypothesized that both child and adult ASD studies would implicate fronto-temporo-parietal networks, but that child and adult ASD studies would show few differences, since many suggest that the neuropathology involved with ASD occurs early in development.

Methods: First, we conducted a PubMed literature search for both adult ("*adult*", "*autism*", "*Asperger*", and "*fMRI*") and pediatric ("*child*", "*autism*", "*Asperger*", and "*fMRI*") populations published through June 2011. These searches were limited to English language publications and humans. Studies were included if they: (a) were original reports of task-dependent fMRI experiments, (b) included ASD and TDC groups, (c) reported between-group differences in neural activation in stereotactic coordinates (Talairach or MNI). Studies were excluded if they were: (a) review articles, (b) only functional connectivity analyses, (c) failed to report either between-group differences or stereotactic coordinates. Eligible studies were then categorized as either pediatric (<18 years old) or adult (equal or greater than 18 years old).

Second, we used GingerALE 2.1 software to transform all eligible studies' data into Talairach space, and then to conduct the ALE meta-analysis. Our focus was two-fold. First, we conducted ANOVAF focus was on developmental comparisons of pediatric vs. adult studies for ASD > TDC and TDC > ASD. We also evaluated these contrasts within pediatric-only and adult-only comparisons. All comparisons used a false-discovery rate (FDR) procedure with 5000 p-value permutations to control for multiple comparisons.

Results: Our search yielded 456 pediatric and 188 adult articles. Of these, 19 pediatric and 25 adult articles met eligibility criteria for ALE meta-analysis. These studies included 566 (278 ASD, 288 TDC) pediatric and 595 (284 ASD, 311 TDC) adult participants. Meta-analyses of child studies showed significantly greater activation in ASD vs. TDC children in regions including the right insula and left superior temporal gyrus (STG; BA 22) and significantly greater activation in TDC vs. ASD children in regions including the left STG (BA 41) and left superior and inferior frontal gyri (BA 10, 9). Meta-analyses of adult studies showed significantly greater activation in ASD vs. TDC adults in regions including the right amygdala and right medial frontal cortex (BA 8), and significantly greater activation in TDC vs. ASD in regions including the left globus pallidus and left amygdala. Integrating the two for a developmental perspective, there were no significant differences between either ASD child or ASD adult studies greater than TDC, or TDC greater than ASD child or adult studies.

Discussion: While preliminary, our results support two positions. First, ASD involves perturbations in fronto-temporal structures compared to TDC across a variety of cognitive tasks. Second, such alterations likely occur early in development given that we failed to identify significant differences between child and adult studies. Further work is warranted to evaluate such discrepancies in neural development between ASD and TDC participants.

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142. Prenatal exposure to Cigarettes or Alcohol affects the Volume of Cerebellum in Attention-Deficit/Hyperactivity Disorder

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Background: Prenatal exposure to nicotine or alcohol increases the risk of Attention Deficit Hyperactivity Disorder (ADHD). To date, studies examining this relationship have used symptom scales as outcome measures, and have not investigated the neurobiological pathways involved. The present study investigates the effects of

self-reported maternal smoking and alcohol use during pregnancy on global brain volumes in children with ADHD and typically developing controls. We compared brain volumes derived from anatomical MRI-scans from subjects with ADHD who had been prenatally exposed to cigarettes or alcohol to those from subjects with ADHD who had not, and to those from typically developing controls with no prenatal exposure. We hypothesized that cerebellum would be preferentially affected, as it shows a protracted developmental course, potentially rendering it more susceptible to the effects of teratogenic substances.
Methods: Participants were selected from a large cohort study of brain development in ADHD. We performed two rounds of individual matching: one where socio-economic status (SES) was a matching criterion and a second where birth weight (BW) was given priority. Matching for SES provided the most parsimonious (if not full) control for confounding issues such as postnatal developmental environment, and maternal nutritional status. The second matching by birth weight was performed to investigate the specificity of the findings to prenatal exposure, by controlling for general prenatal adversity. Subjects were scanned on a 1.5T Phillips MRI scanner. Linear polynomial contrasts were specified to test our main hypotheses.
Results: For prenatal exposure to both smoking and alcohol, we found a pattern where subjects with ADHD who had been exposed had the smallest brain volumes and unexposed controls had the largest, with intermediate volumes for unexposed subjects with ADHD. This effect was most pronounced for cerebellum. Results were similar for groups matched by SES and BW.
Discussion: We conclude that a well-established increased risk for ADHD associated with prenatal exposure to teratogenic substances may be mediated through cerebellum.
Disclosure: P. de Zeeuw: None. **F. Zwart:** None. **R. Schrama:** None. **H. van Engeland:** None. **S. Durston:** None.

143. Functional Magnetic Resonance Imaging of Social Neural Processing in Rats exposed Prenatally to Valproic Acid

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Background: The treatment of pregnant rats on mid-gestational day 12.5 with 600 mg/kg/2.5 mL of valproic acid (VPA) has been shown time and time again to lead to behavioral and neurobiological changes resembling in features reported in autism. Although there are no rodent behavioral models that can reproduce all the features of autism, the VPA model comes quite close to modeling in rats many of the effects that are observed in humans exposed prenatally to the anti-seizure medication. Here, we focus on the effects of prenatal VPA on social neural circuits and social behaviors over the postnatal period.
Methods: Timed pregnant rats were given VPA as indicated above following standard protocols published widely in the literature. Offspring were observed for changes in anogenital distance and weighed at several postnatal periods. They were also tested for isolation-induced emission of ultrasonic vocalizations (USV's). We characterized USV's according into specific categories based on the observed spectral features. In separate cohorts of animals, adolescent play fighting and social interactions were measured. The latter behaviors were also tested in a group of adult rats as well. Once animals reached adulthood, they were prepared for functional magnetic resonance using previously published methods. Animals were acclimated to 5 days of restraint and then imaged for their neural response to the presentation of a non-social and social stimulus. Before imaging, animals were fitted with permanent ICV cannula's for in order to administer 0.9% sterile saline or 125 ng of a selective V1a antagonist. Following the ICV injection, rats were tested on a social interaction test. Time spent sniffing a cage mate was measured. Thirty-to-40 minutes later, rats were scanned on a 7Tesla Bruker USR system using a T2 weighted fast spin echo sequence (TE = 43.2msec, TR = 1562 msec, FOV = 28 mm2 x 1 mm slice, 64 x 64 data matrix).
Results: Although no differences were observed in pup weights from PND 2-24, we did find that VPA rats show elevations in specific types of USV's, complex, downward and chevron, on PND 5 but not PND11. On PND11 we observed reduced upward vocalizations. At the adolescent stage (PND35-40) reduced play behavior was found in addition to lower social interactions. This was observed along with increased cage exploration and no differences in locomotor activity. In addition to signs of social deficits, we observed reduced cognitive performance on a novel object recognition test. Our imaging results indicate that VPA rats show greater neural activation to the social stimulus in the agranular insular cortex and the temporal cortex. Interestingly, blocking V1a vasopressin receptors reduced this effect of prenatal VPA.
Discussion: Our data present overall behavioral and neural adaptations as a consequence of VPA exposure in utero and support a potential role for vasopressin in the changes in neural processing.These observed changes in behavior may correspond to specific actions of VPA prenatally on the development of social neural circuits. On the other hand, our observations of cognitive deficits may indicate that the results are really associated with wider developmental effects at the synaptic level, which has been previously reported. Moving forward, similar studies should be carried out with alternative murine models at higher fields 11.1T.
Disclosure: A. Felix-Ortiz: None. **M. Febo:** None.

144. Disturbed Microstructural Integrity of the Frontostriatal Tracts and Cognitive Dysfunction in Children with Attention Deficit Hyperactivity Disorder

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Background: Evidence from neuroimaging and neurobiological studies suggests that cognitive dysfunction and abnormalities in the frontostriatal tracts may be related to attention deficit hyperactivity disorder (ADHD). However, no previous study has comprehensively examined the correlations between frontostriatal circuitry and a wide range of executive function in ADHD. As the first study to examine the structural connectivity using diffusion spectrum imaging in ADHD, this study aimed to examine the association between attention and executive function and the microstructural integrity of the frontostriatal tracts in children with ADHD.
Methods: The sample included 25 children with ADHD (mean age = 11.36 ± 2.14) and 25 age-, sex-, IQ-, and handedness-matched typically developing children (TD). ADHD diagnosis was based on a structured diagnostic interview by an experienced child psychiatrist. Attention was assessed by the Continuous Performance Test (CPT) and executive function was measured by the Cambridge Neuropsychological Test Automated Battery (CANTAB): Intra-dimensional/Extra-dimensional Shifts (IED), Spatial Working Memory (SWM), and Stocking of Cambridge (SOC).The frontostriatal tracts were reconstructed by Diffusion Spectrum Imaging (DSI) tractography and were subdivided into four functionally distinct segments, including dorsolateral, medial prefrontal, orbitofrontal, and ventrolateral tracts. Tract-specific analysis was used and generalized fractional anisotropy (GFA) values were measured along individual

targeted fiber tracts to investigate alterations in microstructure integrity.

Results: Children with ADHD had lower GFA in the four frontostriatal tracts bilaterally, performed worse in the CPT and CANTAB than TD children. Multiple regression analysis revealed that left and right orbitofrontal tracts were associated with number (R^2, 0.29) and severity (R^2, 0.43) of inattention symptoms in children with ADHD, respectively. Focused attention including omission errors, Hit RT standard error, variability, and perseveration were explained by the integrity of right dorsolateral, left orbitofrontal, and left ventrolateral tracts (R^2, 0.41-0.45) in TD children. Sustained attention indicated by Hit RT and standard errors changed by block were explained by right medical prefrontal, left orbitofrontal and right ventrolateral tracts (R^2, 0.45), and by right dorsalateral and left orbitofrontal tracts (R^2, 0.40), respectively, in TD children. Impulsivity indicated by commission errors and response style were related to left ventrolateral tract in children with ADHD (R^2, 0.17) and to right ventrolateral tract in TD children (R^2, 0.21), respectively. Vigilance indicated by Hit RT inter-stimulus intervals was predicted by left dorsolateral and right ventrolateral tract in children with TD (R^2, 0.33). For the IED task, left orbifrontal and ventrolateral tracts were associated with adjusted total errors (R^2, 0.42, 0.35) and trials (R^2, 0.41, 0.33) in children with ADHD and TD children, respectively. For SWM, left orbitofrontal tract was associated with strategy utilization (R^2, 0.15) and total errors (R^2, 0.23)in children with ADHD; right dorsolateral and medial prefrontal tracts were associated with strategy utilization in children with TD (R^2, 0.31); and right dorsolateral, left orbitofrontal, and bilateral ventrolateral tractswere associated with total errors in children with TD (R^2, 0.47). For SOC performance in children with ADHD, right orbitofrontal tract was associated with number of problems solved in minimum moves (R^2, 0.28); and left orbitofrontal tract was associated with mean initial thinking time (R^2, 0.25). For SOC performance in TD children, right dorsolateral tract was related to total moves (R^2, 0.23) and mean initial thinking time (R^2, 0.21).

Discussion: These findings support previous diffusion tensor imaging studies showing decreased fractional anisotropy in prefrontal white matter and striatum in children with ADHD. The lack of an association between attention performance measured by the CPT and the frontostriatal tracts in children with ADHD may be explained by immaturity of frontostriatal tracts in children with ADHD or by the potential role of other tracts in maintaining attention and vigilance in children with ADHD. However, our results strongly support an association between integrity of the frontostriatal tracts, particularly left orbitofrontal tract, and executive function in both ADHD and TD children.

Disclosure: S. Gau: Part 4: Dr. Gau has conducted clinical trials on behalf of Eli and Lilly Co. **K. Merikangas:** None. **W. Tseng:** None.

145. Corpus Callosum and Anterior Commissural Aberrations in Aggressive Bipolar Youth

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Background: Youth with bipolar spectrum disorders (BPD) exhibit significant levels of aggressive behavior, which contributes to significant functional impairments and less responsiveness to treatment. Thus, the pathophysiology of aggression in pediatric bipolar spectrum disorders (BPD) warrants research focusing on biological markers which can address its etiology and eventually lead to targeted treatment interventions. White matter (WM) has an extremely high level of structural organization that compartmentalizes and restricts water motion, thus, diffusion tensor imaging (DTI) has great potential for investigating WM pathology. The specific neural substrate for BPD is not well established, although it has been suggested that thecorpus callosum (CC) may play a role in the pathogenesis of BPD. Our study acquired high resolution DTI from youth with BPD and compared the DTI data with that of age-matched healthy controls collected as part of another research study. We specifically compared FA values between groups for 5 segments of the CC and the AC. We hypothesized that there would be decreased fractional anisotropy in the CC for the BPD group but did not have a specific hypothesis for the AC.

Methods: This study was conducted at the University of Texas Southwestern Medical Center in Dallas, TX. Participants: Ten youth (mean age 13.9 ± 3.6; 60% female) with BP-I and BPNOS (not otherwise specified), were evaluated utilizing standardized research instruments to confirm diagnoses, assess severity of mood symptoms and illness. The Life History of Aggression (LHA) scale was also obtained. Data from 10 healthy controls (mean age 13.6 ± 3.6; 40% female) obtained in the context of a different DTI study were utilized . The controls had no lifetime history of any psychiatric disorder based on clinical interview.

DTI: *In vivo* human DTI data was acquired using a single-shot echo-planar imaging (EPI) sequence with SENSE parallel imaging scheme (SENSitivityEncoding, reduction factor = 2.5). Anisotropy was measured by calculating FA The tensor fitting and FA calculation were done using DtiStudio. Controls were scanned at the Children's Medical Center of Dallas imaging facilities on a 3T Philips AchievaMR system using the same scanning protocols. Statistical analysis: TBSS was used for voxel-wise comparison. Pearson's correlations (2-tailed) were performed correlating FA values in the CC (segment 1) and AC with total LHA score.

Results: There were statistically significant differences for FA values at CC and AC between the BPD group and controls. The BPD group had significantly lower FA values in the anterior region of the CC ($p = .015$) but no statistically significant group differences were observed in the middle and posterior CC.

There was a significant negative correlation between FA values and LHA scores (anterior region of the CC, $p = .05$; AC, $p = .02$).

Discussion: These findings are important in that they provide additional confirmatory evidence for the role of the anterior region of the CC in the pathogenesis of BPD. Further, we are the first to report WM disruption in the AC in youths with BPD. Finally, the significant correlation of these WM disturbances with aggression in youths with BPD provides additional support for the purported role of the CC and AC in the integration and processing of emotional information. However, additional studies are needed to replicate these findings as we could find no other studies reporting on bipolar youth with aggression, nor were we able to find other reports of a role for the CC or AC in aggression regardless of psychiatric diagnosis.

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146. The Impact of Methylphenidate Treatment on Cortical Activation during Facial Emotion Processing in Children with Dysphoric Mood Dysregulation Disorder

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Background: A significant proportion of children with attention deficit hyperactivity disorder (ADHD) also display profound emotion regulation deficits, resulting in chronic irritability, severe temper outbursts and aggression. In recent years, these children have been variously characterized as having early-onset bipolar disorder, severe mood dysregulation and, now anticipated for DSM-V, dysphoric mood dysregulation disorder (DMDD). Despite high rates of medication use in this population, little is known about effective pharmacotherapies for emotion related symptoms. Methylphenidate (MPH) is a dopamine and norepinephrine acting agent with a known benefit for multiple symptoms domains of ADHD. The neural mechanisms underlying MPH's clinical effects on emotion processing and regulation are largely unstudied in this population. We hypothesize that during a facial emotion matching task, MPH will impact activation in ventral cortical regions associated with emotional control.

Methods: Medication-free, right-handed 10-15 year olds were interviewed using the K-SADS and a customized DMDD interviewto assess for the presence of psychiatric disorders. IQ screening and parent ratings of emotion regulation capabilities were also collected. Youth were treated open-label with the MPH hydrochloride osmotic-release oral system (Concerta^{TM}), titrated to a therapeutic dose (1-1.5 mg/kg/day) over 4 weeks. We acquired fMRI scans on a Siemens 3T scanner using the Hariri facial emotion matching task before and after 4 weeks of MPH treatment. During the task, 3 blocks of faces were interleaved with 4 blocks of control shape stimuli. During the emotion matching condition, subjects selected 1 of 2 faces on the bottom row expressing the same negative emotion (anger or fear) as the target face on the top row. During the control condition, they matched simple geometric shapes (circles, vertical and horizontal ellipses). Each block consisted of 5 different trials presented sequentially for 4.5 seconds each. During the scanning session, a high-resolution 3D magnetization prepared rapid gradient echo (MPRAGE) scan was first acquired. Then, a total of 77 functional image volumes were collected during the 3-minute face task scan (TR = 2.25 s and voxel size of 2.5x2.5x3.5 mm). The fMRI data were pre-processed and co-registered to the MPRAGE anatomical images using AFNI software. A general linear model was utilized to estimate the brain response during face and control blocks. Activation was assessed as the difference between the response to face and control stimulus. For group analysis, each participant's data were aligned to the standardized Talairach atlas. A whole brain voxel-wise analysis was performed using a cluster threshold of $p < 0.05$ (corrected for multiple comparisons) to compare activation between pre- and post-medication scans.

Results: Of the 11 psychotropic medication free participants recruited, imaging data from 2 participants were unusable due to excessive motion and metal artifact from a dental device. The remaining 9 participants (6 males) were aged 9-15 (12.0 ± 1.83) and had full scale IQs in the average range (106 ± 11.68). In addition to ADHD-CT diagnoses, several participants met criteria for disruptive behavior disorders and anxiety disorders. Few side effects were reported and all were mild. All participants tolerated the dose escalation schedule (final mean dose = 56 mg/day). A significant improvement on the parent ratings of the Emotion Regulation Checklist was noted from baseline (64.4) to week 4 (57.0) of treatment ($t = 3.35$, $p = 0.01$). On the facial emotion matching task, participants demonstrated high rates of accuracy when matching shapes (pre: 92% post: 93%) and faces (pre: 83% post: 86%), indicating they were successfully attending to the task. Whole brain voxel-wise analysis revealed significant activation decreases in the left orbitofrontal cortex (cluster peak: [-25, 59, 4], cluster size = 118 voxels) and right cerebellum (cluster peak: [41, -47, -34], cluster size = 139 voxels).

Discussion: We present preliminary data on the neural impact of MPH on facial emotion processing in youth with ADHD and DMDD. In this open-label fMRI study, MPH was well-tolerated when dosed therapeutically and resulted in parent-rated improvements in emotion regulation. Four weeks of MPH use was associated with decreased activation in the orbitofrontal cortex, a region associated with emotional processing, for a faces vs. shapes contrast. Future work in larger samples will include region of interest and functional connectivity analyses to determine the involvement of limbic and striatal networks in this MPH-mediated orbitofrontal activation change.

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147. Decreased Connectivity of the Medial Prefrontal Region With-in the Default Mode Network in Youths with ADHD is Associated with Reduced Attention

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Background: In Attention-Deficit/Hyperactivity Disorder (ADHD), structural and functional neuroimaging studies have implicated regions of the Default Mode Network (DMN) in the pathophysiology of ADHD. While the functions of the DMN are still being clarified, it may play a role in top-down attentional control, with the degree of attenuation of the DMN during task positive events relating to improved attention and task performance. During normative development,stronger connections between anterior medial prefrontal cortex (mPFC) and posterior DMN regions such as the posterior cingulate cortex (PCC) have been found with increasing age.[1,2] In ADHD, a recent study found a reduction in correlation strength between the mPFC and PCC[3], suggesting that delayed or atypical functional connectivity patterns may exist in the DMN in youths with ADHD. The aim of this study is to extend previous findings of an underconnected mPFC region to the DMN in ADHD, by examining whether measures of mPFC connectivity to the DMN are associated with neurobehavioral measures.

Methods: Sixteen adolescents (aged 13.9 ± 2.7 years; females: n = 9 (56%), with DSM-IV ADHD, combined or inattentive type and 22 healthy controls (HC) similar in age (13.5 ± 2.3 years; females = 12 (55%) received MRI scans using a 3T Siemens Trio scanner with a 12-channel head coil. In addition, 8 minute resting BOLD images were obtained for each subject. BOLD echoplanar images (TR = 2.0 s, TE = 28 ms, GRAPPA parallel acquisition with acceleration factor = 2, 40 slices at 3 mm slice thickness, 64 x 64 matrix) were obtained during the resting state, where subjects were instructed to, "Keep your eyes open and remain awake and try to let thoughts pass through your mind without focusing on any particular mental activity." Preprocessing steps included motion correction, coregistration to anatomic, normalization to MNI template, regression of white matter ROI, CSF, and soft tissue time series from each voxel (PSTCor), and low pass filtering (< 0.1 Hz). Six regions in the DMN were identified from data from 1228 subjects from 28 sites (FCON 1000 and ADHD 200 datasets), corresponding to mPFC, PCC, bilateral temporoparietal junction, and bilateral inferior temporal regions[4]. These ROI were utilized to identify regions of the DMN in the current data set of 38 subjects and correlation coefficients between each node's time series were calculated. The Baratt Impulsiveness Scale (BIS) was utilized to measure attention, motor and non-planning constructs of impulsivity. Correlations

were performed between BIS measures and connectivity measures between the mPFC and DMN network.
Results: The mPFC node was significantly less correlated to the rest of the DMN in ADHD subjects ($p = 0.029$, two-tailed t-test) than in control subjects. Significant increases in BIS sub-scale scores (BIS Non-planning, $p < 0.001$; BIS Motor, $p < 0.001$; BIS Attentional, $p = 0.003$) as well as BIS total scores ($p < 0.001$) were found in the ADHD group relative to controls. Within the ADHD subjects, Barratt Inattentiveness scores were significantly higher in subjects with lowest correlation between the mPFC node and remainder of the DMN ($r = -0.54$, $p = 0.037$). Total impulsivity was also negatively associated with connectivity between mPFC and the remaining DMN although this was not significant in our data ($r = -0.43$, $p = 0.11$). No significant relationship was seen between mPFC and DMN connectivity in control subjects.
Discussion: Our results support previous findings of a reduction in mPFC connectivity to other regions of the DMN [3]. Within the ADHD group, inattentiveness scores were significantly higher in youths with the lowest correlation between the mPFC and the remainder of the DMN. These results suggest that atypical development of the DMN may be associated with the impulsive and inattentive symptoms seen in ADHD. Further study of the developmental trajectory of this network in combination with the behavioral manifestations of ADHD is warranted.
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Disclosure: M. Lopez-Larson: None. **J. Anderson:** None. **M. Ferguson:** None. **D. Yurgelun-Todd:** Part 2: Deborah Yurgelun-Todd Consultant: Kyowa Hakko Kirin Perry Renshaw Consultant: Ridge Diagnostics, Kyowa Hakko Kirin, Part 4: Deborah Yurgelun-Todd: Kyowa Hakko Kirin.

148. Assessing Amyloid Load in Non-demented Young Adults with Down's Syndrome

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Background: With PET amyloid (Aβ) imaging, much has been learned about fibrillar Aβ deposition in the living human brain, such as: 1) cortical Aβ in Alzheimer's disease (AD) can be twice that of cognitively unimpaired controls (CON) but similar in "Aβ-spared" areas (e.g., white matter, cerebellum); 2) Aβ deposition occurs in 20-30% of CONs; and 3) Aβ deposition begins in striatum of presenilin-1 early-onset familial AD (eoFAD) mutation carriers [1,2]. Individuals with Down syndrome (DS) are at high risk for AD because of an extra copy of chromosome 21 (codes for Aβ precursor protein) and over 40% exhibit AD symptoms by 50-59 years of age [3,4]. Post-mortem studies report AD pathology in 60-90% of DS adults that increases with age [5]. The robust *in vivo* detection of fibrillar Aβ requires correction of the PET signal for nonspecific uptake that is based on estimates of nondisplaceable radiotracer retention in a reference region. The cerebellum (CER) is often used as reference because negligible levels of fibrillar Aβ are found in CER in sporadic AD. However, significant CER Aβ deposits can be a common pathologic finding for other groups, such as eoFAD [2]. The pons (PON) region lacks Aβ and is an alternate reference but this white-matter rich area has substantial nonspecific signal and may be not be the best representation of nondisplaceable uptake in Aβ-rich areas. Goal: To evaluate and relate [C-11]PIB retention measures obtained in non-demented young DS subjects, using either CER or PON as reference region.
Methods: [C-11]PIB PET imaging (HR+ scanners, 10-15 mCi, 50-70 min post-injection scan) was performed for 35 DS subjects (38 ± 6 years,18 Female) who also underwent MR imaging for region (ROI) definition and CSF dilution correction of the PET data. ROIs included 5 primary cortical areas (anterior cingulate (ACG), frontal, parietal (PAR), lateral temporal, precuneus), a global cortical mean (CTX5), anterior-ventral striatum (AVS), CER, and PON. Summed tissue uptake was computed for each ROI (SUV: scaled to injected dose, body mass) and tissue ratios were calculated using either CER (SUVRcer) or PON (SUVRpon) as reference. Subjects were deemed PiB(+) if a retention measure exceeded 1.5 SD above the mean (93% percentile) ROI SUVR value.
Results: The CER SUV (0.64 ± 0.19) was nearly 1.5-fold less than that for the white-matter rich PON (1.00 ± 0.24). Cortical SUVRcer ranged from 1.38 ± 0.32 (PAR) to 1.55 ± 0.42 (ACG), while SUVRpon ranged from 0.89 ± 0.19 (PAR) to 1.00 ± 0.28 (ACG). Similarly, CTX5 SUVRcer was 1.46 ± 0.36 and SUVRpon was 0.94 ± 0.23. Six (17%) DS subjects were PiB(+) across the cortical SUVRcer values, while only 3 were PIB(+) by the CTX5 SUVRcer. Fewer DS subjects were PiB(+) by SUVRpon (4 PiB(+) across cortex and 3 PiB(+) by CTX5). There was 100% overlap between the SUVRpon PiB(+) and SUVRcer PiB(+) groups. Several (3-5) more subjects were near the 1.5 SD threshold. In terms of the striatum, 5 PiB(+) DS subjects had striatal SUVRcer retention that was more than 1.5 SD above the AVS ROI mean (1.48 ± 0.58); this was also true for the 4 SUVRpon PiB(+) subjects.
Discussion: Nearly 20% of the young non-demented DS subjects were PiB(+). Deposition of Aβ was clearly evident in the striatum of DS subjects, consistent with significant striatal Aβ in eoFAD. This focal striatal similarity is interesting because these early onset forms of AD share overproduction of Aβ (particularly Aβ1-42), rather than impaired clearance that is suspected in late-onset. The data also indicate reduced sensitivity for the detection of early Aβ deposition with the use of pons as reference or the use of a global cortical retention measure rather than inspection of retention in individual cortical areas (consistent with findings in AD-focused studies). More PiB(+) DS subjects were detected with SUVRcer (relative to SUVRpon) indicating that CER Aβ was not significant for this DS group. The PiB(+) subjects identified by SUVRpon were also PiB(+) by SUVRcer. When PON is required as reference, these results may differ from those obtained using CER; this likely results from the limited dynamic range of the SUVRpon measure.
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149. Altered Functional Connectivity in Boys and Girls with Attention Deficit Hyperactivity Disorder

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Background: Attention-deficiency hyperactivity disorder (ADHD) is more frequent among boys than girls, and is characterized by

symptoms of inattention and impulsivity and deficits in dopamine (DA) neurotransmission are implicated in its neuropathology. However the nature of the brain function deficits in ADHD is still incompletely understood. The aim of the present study was to take advantage of large resting functional connectivity datasets from children with ADHD as well as from typically developing children (TDC) to investigate changes in network architecture in ADHD. Based on previous neuroimaging findings we hypothesized that ADHD will be associated with abnormal connectivity in regions that are part of "hot" motivational (ventral striatum and orbitofrontal cortex, OFC) and of "cool" attention (parietal cortex) networks that would correlate with severity of ADHD symptoms.
Methods: "Resting-state" magnetic resonance imaging datasets from 255 ADHD (11.2 ± 2.5 years old; 215 boys) and 316 TDC (11.2 ± 2.5 years old; 168 boys) from four research sites around the world (Baltimore, New York, Portland and Peking) of the open data-sharing "ADHD-200" initiative were included in the study. Functional connectivity density mapping (FCDM) with parallel computing was used to speed up the calculation of short- and long-range FCD maps at 3-mm isotropic resolution. Seed-voxel correlation analysis was used to assess the functional connectivity patterns of the "hot" motivational and "cool" attention regions identified by voxelwise ANOVA (with age and gender covariates). Statistical significance was set at $P < 0.05$, family-wise error corrected for multiple comparisons in the whole brain. Significant clusters identified by the SPM analysis were subject of subsequent regions-of-interest analyses (ROI). Average values of short- and long-range FCD in ROI cubic volumes (0.73 cc, 27 voxels) were computed. These ROI measures were correlated with ADHD symptom scores (ADHD index, inattention and impulsivity) and used to test for gender differences (t-test and ANOVA).
Results: Children with ADHD had 15 ± 2% higher short-range FCD in ventral striatum, caudate and orbitofrontal cortex and 33 ± 4% lower long-range FCD in superior posterior parietal cortex than controls. These functional abnormalities were correlated with symptoms of inattention and impulsivity across subjects ($P<0.001$). Seed-region correlation analyses demonstrated the higher connectivity of the motivational networks with the OFC, the lower connectivity of the default-mode network with ventral striatum and OFC, and the lower connectivity of the superior parietal cortex in ADHD compared to TDC. The ROI analyses demonstrated that the higher short-range FCD in OFC for ADHD compared to TDC was pronounced for boys ($P<0.001$) but not for girls; for the ventral striatum, however, both genders demonstrated higher short-range FCD in the ADHD group compared to TDC ($P < 0.05$). The long-range FCD in the superior parietal cortex, lower for ADHD than for TDC for boys ($P < 0.001$) but not for girls, showed a gender × diagnosis interaction effect ($P = 0.008$; ANOVA).
Discussion: The higher proportion of short-range FCD in "hot" motivational networks in ADHD children is consistent with lower dopaminergic function in reward-motivation pathways in ADHD. Since DA is a neuromodulator that changes the efficacy of other transmitter signals by reducing spontaneous background activity, lower dopaminergic function might cause larger higher spontaneous activity and hence increased local functional connectivity in ADHD. Since we have shown that brain activity in the superior posterior parietal cortex was correlated with the availability of DA transporters in striatum, lower dopaminergic function in ADHD patients could impair brain activity and the long-range connectivity of the superior posterior parietal cortex, a region that includes prominent long-range functional connectivity hubs. The lower connectivity of the superior posterior parietal cortex with dorsal attention network regions (dorsal precuneus and superior parietal cortex) for ADHD than for TDC further supports this interpretation. Since abnormal connectivity in "hot" motivational (OFC) and "cool" attention (superior parietal) regions was pronounced for ADHD boys but not for ADHD girls, these findings might help explain the higher prevalence of ADHD among boys than girls.
Disclosure: D. Tomasi: None. **N. Volkow:** None.

150. Reduced Insular Volume in Attention Deficit Hyperactivity Disorder

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Background: Inattention, impulsivity and hyperactivity are core symptoms of Attention-Deficit Hyperactivity Disorder (ADHD). Current studies have focused on evaluating prefrontal-striatal circuits in ADHD, which includes the anterior cingulate (ACC). The insula has been associated with emotional awareness, risk, uncertainty and anticipation, attention, perceptual decision-making, cognitive control and performance monitoring [1]. Despite the wide array of cognitive functions associated with the insula, few studies have evaluated this structure in ADHD. Furthermore, the insula and ACC have been found to co-activate on numerous functional imaging studies including those studying goal-directed attention and emotion and both are crucial structures in the salience network.[1, 2] The aim of this study was to evaluate whether structural differences in the insula and ACC co-exist in adolescents with ADHD compared to healthy controls (HC) and to determine if structural changes correlate with measures of attention and impulsivity.
Methods: Sixteen adolescents (aged 13.9 ± 2.7 years; females: n = 9 (56%)), with DSM-IV ADHD, combined or inattentive type and 22 HCs (13.5 ± 2.3 years; females = 12 (55%)) received MRI scans on a 3T Siemens Trio scanner including a T1-weighted 3D MPRAGE grappa sequence acquired sagittally (TE/TR/TI = 3.37 ms/2.0s/1.1 s, 256x256 acquisition matrix, 160 slices, 1.0 mm slice thickness). Cortical reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/). Cortical thickness (CT) and volumetric data for the insula and ACC were extracted and UNIANOVA's were performed for the average CT and volume of the insula and ACC, covarying for sex and intracranial volume (ICV) (for volumes only). Anterior insula was defined as three insula regions including the insular short, sulcal central insula, and the sulcal circular insula anterior. The posterior insula was defined as the sulcal circular insula superior, the sulcal circular insula inferior, and the gyral insular long. The Barratt Impulsiveness Scale (BIS) was utilized to measure constructs of impulsivity. Pearson's correlations were performed between BIS measures and ACC and insular regions found to be significantly different between ADHD and HC.
Results: Our findings indicate that ADHD youths had smaller left ($p = 0.01$) and right ($p = 0.029$) anterior insular volumes compared to HC despite no significant between group differences for total brain volume, ICV, or bilateral ventricles. No CT differences were found for any region in the anterior insula between HC and ADHD youths. Univariate Analyses of ACC volumes utilizing ICV as a covariate found that ADHD youths showed a trend toward smaller volumes in the right caudal ACC ($p = 0.08$). Furthermore ADHD youths were found to have reduced CT in the left ($p = 0.02$) and right ($p = 0.02$) rostral ACC. Significant increases in BIS sub-scale scores (BIS Non-planning, $p < 0.001$; BIS Motor, $p < 0.001$; BIS Attention, $p = 0.003$) as well as BIS total scores ($p < 0.001$) were found in the ADHD group relative to HC. The right anterior insula volume was significantly correlated with BIS Non-planning scores ($r = -0.54$, $p = 0.04$) in the ADHD group whereas ACC volumes and CT measures did not correlate with any BIS measures.
Discussion: To our knowledge this is the first report of a bilateral reduction in insular volumes in ADHD youths. Our findings of reduced CT and volume in the ACC in youths with ADHD is consistent a prior study in youths with ADHD[3]. Further our data showed that right anterior insula volume was negatively correlated with BIS Non-planning measures in the ADHD group. Together the anterior insula and ACC form a "salience network" that functions to segregate the most relevant stimuli in order to guide behavior [2]. Furthermore, the right anterior insula is thought to be important in the switching off and on of other large-scale networks such as

the attention network and default mode network, to facilitate access to attention and working memory [2]. Our findings suggest a role for the insula in modulating attentional capacity in ADHD.

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151. Diffusion Imaging in Individuals with Partial Deletions of the Williams Syndrome Critical Region

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Background: Williams syndrome (WS) is a rare neurodevelopmental disorder resulting from hemizygous microdeletion of ~25 genes on chromosome 7q11.23. Individuals with WS usually exhibit mild to moderate intellectual disability and pronounced difficulty on tests of visuospatial construction. Prior Diffusion Tensor Imaging (DTI) studies have established that WS is associated with altered white matter (WM) integrity. Studying individuals with partial deletions (PD) in the WS chromosome region (WSCR) could provide insight into the role of a smaller set of genes within that region.

Methods: Twelve individuals (9 females; mean age = 35.3 ± 13.8 (SD) years) with PDs in the WSCR participated in this study. Participants had IQs within the normal range (mean = 96.9 ± 10.8), cognitive profiles consistent with the WS pattern, and varying 7q11.23 deletions which included the elastin (ELN) and LIM-domain kinase 1 (LIMK1) genes; none of the deletions included GTF2IRD1 or GTF2I. Five participants had deletions of only ELN and LIMK1. Twelve healthy individuals matched for age (mean = 35 ± 12.2 years), IQ (mean = 99.6 ± 8.5), and gender (7 females) served as the control group. Diffusion Weighted Images (DWI) were acquired on a GE Signa 1.5T Scanner (2x2x2 mm resolution, 120 gradient directions, b-values between 0 and 1200). DWIs were corrected for head movement and eddy currents using TORTOISE [Pierpaoli *et al*., 2010]. Fractional anisotropy (FA) maps were derived using TORTOISE and were registered in a common space with Tract Based Spatial Statistics (TBSS [Smith *et al*., 2006], part of FSL [http://www.fmrib.ox.ac.uk/fsl/]). Also radial (RD) and longitudinal diffusivity (LD) were analyzed. Nonparametric statistical tests were performed using FSL's randomise procedure with 2000 permutations, using threshold free cluster enhancement (TFCE) for family-wise error corrections of multiple comparisons over the whole brain. In addition, we ran probabilistic tractography from a sphere (20 mm diameter) located around the intraparietal sulcus, where optimized VBM analysis had shown a loss of gray matter volume in the PD group as compared to controls. We binarized the tracts, transformed them into MNI space, and compared paths in PD and healthy controls with TFCE.

Results: We observed significant reductions of FA throughout the brain in PD individuals relative to controls (Fig. 1). 71% of the voxels in 48 major tracts were significant at a threshold of $p < 0.01$, with peaks of significance in the right cingulum bundle, right external capsule and left internal capsule, which contained the most significant voxel (MNI coordinates: x = -40,y = -38, z = -3). RD was increased in PD as compared to controls, although less significantly than FA, but with a similar diffuse pattern. LD did not differ significantly across the groups. Participants with PD in the WSCR were significantly more likely to have fiber paths passing through the superior longitudinal fasciculus than were healthy controls ($p < 0.05$).

Discussion: Reductions in FA accompanied by increases in RD and no change in LD may suggest changes in myelination or possibly fasciculation of axons in major tracts. The alteration in fiber paths emanating from the intraparietal sulcus would support the latter hypothesis.

Our results suggest that alterations in WM integrity in WS are related to a subset of genes in the WSCR. Since LIMK1 and ELN are the only deleted genes common to our entire PD group these findings implicate these two genes in particular. More work is necessary to understand the impact of LIMK1 and nearby genes on WM structure as measured by DTI.

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152. Functional and Anatomical Connectivity Underlying Vulnerability to Auditory Hallucinations in Schizophrenia Spectrum Disorders

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Background: Auditory hallucinations (AH) are a cardinal feature of schizophrenia (SZ) and other psychotic disorders. While a consistent finding in SZ patients with AH has been volume reductions of the superior temporal gyrus (STG), the literature suggests that abnormalities underlying AH are not confined to a single locus but involve distributed brain regions. The "dysconnectivity" hypothesis, first proposed by Wernicke, suggests that SZ arises from abnormal interactions between brain regions. In this study, we used resting state fMRI (rsfMRI) and diffusion tensor imaging (DTI) to assess both functional and anatomical connectivity underlying vulnerability to AH.

Methods: We studied 3 groups: SZ, schizoaffective, or schizophreniform patients with AH (n = 27), those with no history of AH (NAH; n = 14), and healthy controls (HC; n = 28). Patients were stably medicated and recruited from inpatient and outpatient services at McLean Hospital. Participants were 18-65 years old (AH 40 ± 11; NAH 37 ± 10; HC 37 ± 9 years), with no substance abuse in the past 3 months, no significant medical or neurologic disease, and no electroconvulsive therapy in the previous year. We used the Psychotic Symptom Rating Scale (PSYRATS) to collect information about patients' AH.

We acquired a 10 min resting state fMRI scan (TE/TR 24/2500 ms, 42 slices, voxel size 3.5 mm3 isotropic) using a Siemens 3T Trio MR scanner. We performed a seed region analysis of low frequency spontaneous oscillations using FSL version 4.1.6. We placed a

ROI (BP_{ND})	Caudate	Putamen	Pallidum	Substantia Nigra (SN)	Thalamus	Amygdala	Ventral Striatum	SN/Striatum
HC Mean (SD)	1.95 (.34)	2.54 (.41)	3.48 (.61)	1.14 (.30)	0.41 (.08)	.30 (.08)	3.63 (.81)	.50 (.10)
CD Mean (SD)	1.78 (.56)	2.37 (.36)	3.23 (.78)	1.38 (.52)	0.42 (.09)	.57 (.38)	3.44 (.89)	.68 (.26)

10 mm sphere on the left primary auditory cortex (PAC), located in the STG. Coordinates (-42, -26, 10) were identified using probabilistic maps of the PAC (Rademacher *et al*.,, 2001). The time course from the left PAC was entered into a general linear model to identify temporally coherent brain regions. The resulting whole-brain statistical maps were entered into a higher level analysis with the 3 groups, using a statistical threshold of $p < 0.001$, uncorrected.
DTI images (12 directions, 4 averages, b-value 1,000s/mm^2, TE/TR 91/6600 ms, 42 slices, voxel size 1.75 x 1.75 x 3.5 mm3) were acquired in the same session as the rsfMRI scan. Voxel-wise statistical analysis of the fractional anisotropy (FA) data was performed using tract-based spatial statistics (TBSS) in FSL. In addition to whole brain analysis, we performed an ROI analysis guided by the rsfMRI findings. After applying a threshold of $p < 0.05$, uncorrected, to the AH-NAH contrast from the whole brain analysis, we looked for clusters in fiber tracts known to project to the cortical areas found to differ between AH and NAH in the rsfMRI analysis. This process identified clusters in the right anterior cingulum (CIN) and left arcuate fasciculus (AF). Within these clusters, we extracted mean FA values for the 3 groups.
Results: We found that AH patients have reduced functional connectivity between the left PAC and the mediodorsal thalamus (MDT) compared to both NAH and HC groups. Furthermore, connectivity between left PAC and right dorsal anterior cingulate cortex (dACC) was found to covary with AH severity. Whole brain FA analysis yielded no clusters surviving a threshold of $p < 0.05$, FWE-corrected. However, ROI-based DTI analysis, guided by our rsfMRI findings, showed that FA values are higher in the AH group vs. the NAH group in the left AF (AH 0.32 ± 0.04, NAH 0.28 ± 0.05; $p = 0.009$, one-tailed) and right CIN (AH 0.39 ± 0.05, NAH 0.36 ± 0.03; $p = 0.03$, one-tailed). There was no significant difference in FA values when comparing the AH group to the HC group in either region.
Discussion: Patients who are prone to AH appear to have reduced functional coupling between left PAC and MDT. There is also greater coupling between left PAC and right dACC with greater AH severity. The dACC is an area involved in error monitoring. The MDT, which is reciprocally connected to the ACC and other prefrontal regions, plays a central role in cognition and emotion, and has been shown by several groups to have reduced total neuron number in SZ. DTI data from the same patients suggests that patients who are AH-prone may also have anatomical abnormalities involving the right CIN and left AF. The CIN interconnects limbic structures, including cingulate cortex and temporal regions, and is involved in emotional processing and executive control. The AF links the temporal lobe to the prefrontal cortex, and is involved in language processing. Abnormalities of functional connectivity involving the left PAC, MDT, and dACC may have some anatomic basis in the CIN and AF. Dysfunction in circuits involved in error monitoring, emotional processing, and speech/language processing may contribute to AH vulnerability.
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153. Subcortical D3/D2 Receptor Binding in Cocaine Dependent Humans

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Background: Evidence from animalmodel and postmortem human studies points to the importance of dopamine D3 in cocaine dependence (CD). This has yet to be verified via *in vivo* human studies. The objective of this study is to use the D3 preferred radioligand PHNO to compare healthy control (HC) with CD subjects in relevant subcortical regions.
Methods: 10 medically healthy, non-treatment seeking CD subjects (mean age 42 ± 7) were compared to 17 HC subjects (mean age 30 ± 10) with no history of cocaine/illicit substance abuse. After an acclimatization period on an inpatient unit CD subjects received a MRI and then underwent PHNO acquisition using a High Resolution Research Tomograph (HRRT) for purposes of quantifying brain D3/D2 binding potential (BP_{ND}). Subjects received a bolus injection of 316 ± 140 MBq of [^{11}C] PHNO with a total injected mass of 0.028 ± 0.004 μg/kg. The specific radioactivity was 63 ± 29 MBq/nmol at the end of synthesis and 35 ± 17 MBq/nmol at injection time. Parametric images were computed using the simplified reference tissue model (SRTM2) with the cerebellum as the reference region.
Results: Subcortical regions rich in D2 and D3 were chosen for region-of-interest (ROI) analyses. No statistically significant changes were seen between HC and CD subjects in the caudate (–9%), pallidum (-7%), putamen (-7%), substantia nigra (22%), ventral striatum (-5%) and the thalamus (1%). Statistical significance differences were seen, however, in the amygdala (91%, P = .001) and when the ratio of D3 to D2 areas (SN/Striatum) were compared (30%, P = .02).
Discussion: The results suggest that D3 is important in the amygdala, where it is known to enforce the rewarding properties of cocaine in animals.In addition, D2 rich regions (caudate, putamen, pallidum and ventral striatum) were down-regulated and a D3 rich area (substantia nigra) was up-regulated in cocaine subjects. Further studies are necessary to confirm these encouraging results.
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154. Inhibition, Body Mass Index, and Eating Behavior Across the Life Span

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Background: The prevalence of obesity has more than doubled in children and adults since 1970 (1). Prior research has shown disnhibition in adults (2,3) and in youth (4) to be involved in the regulation of caloric intake with several regions of the brain including the hypothalamus (5) and, according to more recent work, cerebral cortex involved in the control of ingestive behavior. Few studies to date have looked at youth and adults with the same assessments to determine neural and behavioral correlates of

eating behavior and obesity. We examined a sample of 203 healthy volunteers across the age span to examine correlates of BMI, ingestive behavior and disinhibition to examine developmental trends in response inhibition and its relationship to food choice and obesity.

Methods: 157 adults (18 years old and older) and 46 children (less than 18 years old) received anthropometric assessments and a multi-item breakfast test meal following an 8-hour fast as part of a larger battery. Self-report visual-analog scales (VASs) assessed fullness, hunger, and abdominal discomfort before and after eating. Information on response inhibition (Stroop color word) and meal content were recorded.

Satiety quotients ($SQ = (VAS_{pre-meal}\text{-}VAS_{post-meal})$/Total calories) were calculated for hunger, fullness, and abdominal discomfort. Pearson correlations were performed to examine the association between anthropometric measures (BMI and body surface area [BSA]), test meal food preference, levels of satiety, and response inhibition.

Results: Children were evenly divided across gender (% male = 50%), and ranged in BMI z-score from -2.86-2.32 (mean = -0.28 ± standard deviation = 1.460), BMI (kg/m^2): 13.0-33.1 (19.941 ± 5.084), BSA (m^2): 0.64-2.18 (1.424 ± 0.36) and age (years): 4-17 (12.17 ± 3.60). Males comprised 60% of the adult sample, with BMI ranging from 16.3-49.1 (27.0 ± 5.41), BSA: 1.39-2.70 (1.932 ± 0.26), and age: 18-85 (41.54 ± 18.04). Gender was distributed evenly across BMI groups (lean, overweight, obese) for each age group; in youth parental socioeconomic and in adults self socioeconomic status was not significantly different between BMI groups. In youth BSA was positively correlated with proportion of calories from fat consumed ($r = 0.311$, $p = 0.045$). In female youth body surface area was positively correlated with proportion of calories from fat ($r = 0.701$, $p < 0.001$, $n = 22$). No significant correlations were seen in males.

In adults BMI was significantly negatively correlated with Stroop color word score ($r = -0.264$, $p = 0.001$). This relationship only trended in significance for males ($r = -0.202$, $p = 0.051$), while in females both BMI ($r = -0.346$, $p = 0.006$) and BSA ($r = -0.427$, $p = 0.001$) were significantly negatively correlated with Stroop color word score.

Discussion: In our sample the relationship between response inhibition, measured by Stroop color word inhibition score and BMI strengthened with age, suggesting the increasing import of frontal development and inhibition on eating behavior. This trend was strongest in females. Youth differentiated from adults in the relationship between body surface area (a BMI proxy that takes into account difference in size) and fat content of meal. Neural correlates of inhibition and its relationship to eating behavior and obesity should help guide development of interventions for weight gain.

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155. Acute Cortisol Elevations Bias Memory Formation in a Negative Direction only in Depressed Individuals with a History of Early Loss

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Background: In adult rats, history of low maternal care biases learning toward threatening contexts, and alters the effects of corticosteroids on neuroplasticity in the hippocampus. In humans, depressed adults with a history of adversity show greater dysregulation in cortisol than do depressed adults without history of adversity. However, it is unknown whether the effects of cortisol on memory formation in humans vary as a function of the early environment. We hypothesized that history of early loss in depressed adults would moderate the effects of cortisol administration on formation of negatively biased memories.

Methods: In a repeated measures design, eighteen depressed adults (10 women) received 15 mg oral hydrocortisone (CORT) or placebo (order of administration double-blind and counterbalanced) on two different days separated by 48 hours. One hour after drug administration, participants performed a memory encoding task for positive and negative words. Free recall for words was tested several days later, and memory bias was calculated (positive minus negative words recalled) for words encoded on each the CORT and the placebo days. None of the participants experienced early abuse or neglect, but eight of the depressed adults had experienced early loss due to parental divorce.

Results: An increase in negative memory bias for words encoded on the CORT (vs. placebo) day was found for participants with history of loss, but CORT did not alter memory bias in participants without history of loss, $F(1,16) = 6.52$, $p < .03$. No differences were found for perceived stress, depression severity, or at-home endogenous cortisol for participants with vs. without early loss, p's > 0.29. Furthermore, the moderating effects of early loss on CORT's effects on memory bias remained significant after first accounting for these variables, p's $< .05$.

Discussion: Thus, acute cortisol elevation caused an increase in negative memory bias in depressed participants with a history of early loss due to parental divorce, but cortisol elevation did not alter memory bias in individuals without history of loss or adversity. These findings in depressed adults replicate animal literature showing that inter-individual differences in early life experience moderate corticosteroids' effects on learning. In addition, the results further substantiate research suggesting that history of early adversity vs. no adversity engender distinct depressive subtypes, especially with regard to hypothalamic pituitary adrenal functioning. Possibly, early adversity causes long-lasting sensitization of neural processes associated with threat-related learning, and acute cortisol elevation may prime threat-related learning in those with a history of early loss. The findings have potential implications for treatment development, as 1) the positive learning context of psychotherapy appears to enhance treatment response in depressed individuals with history of adversity or loss, and 2) corticosteroid receptor ligands show promise as pharmacological augmentation strategies in treatment for depression. It should be noted that the current findings come

from a small sample without a matched control group, and therefore must be replicated.
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156. Detection of Depression in a Clinical Population With Comorbid Pain Using a Multi-Analyte Biomarker Approach
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Background: Epidemiologic studies indicate that depression is a common comorbidity accompanying chronic pain states. Emerging evidence suggests there are pathophysiologic mechanisms that underlie the coexistence of depression and chronic pain states. A biomarker panel and algorithm for depression diagnosis has been developed consisting of ten (10) biomarkers associated with the neurotrophic, metabolic, inflammatory or HPA axis pathways. Clinical validation in academic and general practice settings showed discrimination of patients with depression from normal controls with p values $= < 0.00001$ and a clinical sensitivity and specificity of circa 90% and 87% respectively. Since comorbid depression can complicate the presentation, clinical course, and response to treatment of patients with chronic pain, our objective was to determine whether this blood-based test could identify depression comorbid with chronic pain.
Methods: Patients from community psychiatric practices were enrolled based upon the clinician's discretion. The majority of patients had recurring depressive symptoms and/or were patients who were difficult to manage or non-compliant. The study enrolled a total of 98 patients, 80 of which (81% compliance) had a blood sample drawn and serum prepared. The mean age of the study population was 48.5 ± 12.6 with a range from 21-80 years. Fifty-six (70%) were female and 24 (30%) were male. An MDD score, reflecting the probability of MDD, was determined by quantitative immunoassay and a proprietary algorithm on the patient samples.
Results: While 16 of 80 subjects had no comorbidity listed, the majority of the enrolled subjects had a variety of co-morbidities, with some of the most common being: arthritis, diabetes, hypertension, obesity and chronic pain. Eighteen of 80 patients had chronic pain comorbid with depression. Six of 18 had chronic pain as the only comorbidity. The remaining 12 had pain and arthritis (8); pain and obesity (3) pain and hypothyroid (1). The MDD scores for depressed subjects with pain and at least one other comorbidity were consistent with a high probability of MDD. Interestingly, of the six patients with only chronic pain: four had a high likelihood of depression of $>90\%$ and two had a low likelihood (10 and 33%).
Discussion: While comorbid depression can complicate the presentation, clinical course, and response to treatment of patients with chronic pain: a blood-based test is able to identify a fingerprint of depression in patients with comorbid chronic pain.
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157. Lack of Stress Reactivity to an Extended Continuous Performance Task in Symptomatic Outpatients with Bipolar Disorder: A Pilot Study
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Background: Serial blood or saliva sampling indicates that adrenal steroids are generally increased in bipolar disorder (BD). Moreover, it is now well established that conditions characterized by elevated corticosteroid concentrations often involve a significant degree of attentional impairment as well. However, the relationship between on-line changes in corticosteroid secretion and attentional performance in BD is unknown. With this consideration in mind, the aim of the present study was to examine the degree of stress reactivity, as measured by serially sampled salivary cortisol, to an extended continuous performance task (CPT) in BD. Relative to a healthy comparison (HC) group, which was expected to have normal adrenal steroid levels and good sustained attention performance, we predicted that a BD group would have (1) an increased basal cortisol level, (2) an exaggerated cortisol response to the CPT, (3) a sustained attention decrement over time, and (4) inverse cortisol/performance relationships.
Methods: Symptomatic but otherwise stable outpatients with BD and HC participants were identified from ongoing studies within the Division of Bipolar Disorders Research. Participants received a medical history, the Structured Clinical Interview for DSM-IV Axis I Disorders - Patient Edition (SCID-I/P) and several symptom severity rating scales. Participants then performed a 30-min long CPT to assess sustained attention. The increased length and difficulty of the task was expected to provoke hyperarousal/stress over the course of the vigil. Participants were required to respond as quickly and accurately as possible to a rarely occurring target stimulus in the context of frequent non-target stimuli. Adrenal steroid levels were measured utilizing immunoluminescent assays on saliva obtained by passive drool sampling. Saliva samples were collected at 5:00 (just prior to cognitive testing), 5:15 (half way through testing), 5:30 (just after testing), 5:45, 6:00, 6:15, and 6:30. Upon completing saliva sampling, participants rated self-perceived effort exerted over the full CPT vigil.
Results: The two study groups were well-matched on demographic variables. There were no statistical differences in basal cortisol levels (the pre-task saliva sample) or baseline attentional performance (the first 10-min of CPT performance) between the groups. However, while the HC group had an elevated cortisol response during the CPT relative to baseline (i.e., the expected cortisol response), the BD group had no cortisol response to the CPT. Moreover, while the HC group evidenced consistently high levels of attentional performance over the 30-min CPT vigil, the BD group had a sustained attention decrement over time. Despite these mean differences between the groups, relationships between cortisol levels and CPT performance did not reach statistical significance. Effort ratings were moderate to high and did not differ between the study groups.
Discussion: This pilot study suggests that individuals with BD have a distinct lack of adrenocortical reactivity to stress during lengthy CPT vigils as measured by serial cortisol sampling in saliva. Although a lack of stress reactivity to the task might be expected to benefit cognitive performance, instead the BD group demonstrated a significant sustained attention deficit. This cognitive deficit did not appear to result from task avoidance, as both study groups performed within acceptable limits and effort ratings did not differ between the groups. Contrary to our predictions, perhaps additional effort is needed for individuals with BD to initiate a stress response, which might in turn help overcome attention

deficits. The present results suggest that even mildly stressful tasks can be accompanied by abnormal neuroendocrine function and accompanying cognitive deficits. Although more anxiety provoking tasks, such as the Trier Social Stress Test, have been sufficient to elicit a cortisol response in BD, the lack of a response to a day-to-day stressor – the need to sustain attention – may help explain this deficit, at least in part, and warrants further study. In that relationships between cortisol and performance levels did not achieve statistical significance in this small-scale study, larger samples may help achieve acceptable statistical power to uncover neuroendocrine/performance relationships in bipolar disorder.

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158. Low CSF Oxytocin reflects High Intent in Suicide Attempters

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Background: Data from animal studies suggest that oxytocin is an important modulating neuropeptide in regulation of social interaction. One human study has reported a negative correlation between CSF oxytocin levels, life history of aggression and suicidal behaviour. We hypothesized that CSF oxytocin levels would be related to suicidal behaviour, suicide intent, lifetime interpersonal violence and suicide risk.

Methods: 28 medication free suicide attempters and 19 healthy volunteers participated in this cross sectional and longitudinal study. CSF and plasma morning basal levels of oxytocin were assessed with specific radio-immunoassays. The Beck Suicide Intent Scale (SIS), the Freeman scale and the Karolinska Interpersonal Violence Scale (KIVS) were used to assess suicide intent and lifetime violent behaviour.All patients were followed up for cause of death. The mean follow-up was 21 years.

Results: Suicide attempters had lower CSF oxytocin levels compared to healthy volunteers. In suicide attempters CSF oxytocin showed a significant negative correlation with the planning subscale of SIS.CSF oxytocin showed a significant negative correlation with suicide intent, the planning subscale of SIS and Freeman interruption probability in male suicide attempters. Correlations between plasma oxytocin levels and the planning subscale of SIS andFreeman interruption probability were significant in male suicide attempters. Lifetime violent behaviour showed a trend to negative correlation with CSF oxytocin. In the regression analysis suicide intent remained a significant predictor of CSF oxytocin corrected for age and gender whereas lifetime violent behaviour showed a trend to be a predictor of CSF oxytocin. Oxytocin levels did not differ significantly in suicide victims compared to survivors.

Discussion: CSF oxytocin may be an important modulator of suicide intent and interpersonal violence in suicide attempters. These data indicate that the brain oxytocin system which is linked to social interaction/attachment and stress protection may exert protective effects in subjects expressing suicide risk.Our finding of a correlation between low CSF oxytocin and high suicide intent may have implications for suicide prevention.

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159. Modulation of Adult Hippocampal Neurogenesis through HPA Axis Activity Determines the Divergent Effects of Distress and Eustress on Affective Disorders

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Background: In animals, complex environments and physical exercise have been shown to reduce depressive behavior, increase hippocampal neurogenesis, and improve cognitive function. Previously, we showed that environmental enrichment facilitated the recovery from social defeat stress and enhanced stress resiliency. In a social conflict paradigm, repeated social defeat stress consistently yields a submissive phenotype and is an ethological means of inducing depression in mice. Social defeat stress (SD) and enriched environments (EE) are both strong modulators of hypothalamic-pituitary-adrenal (HPA) axis function. However, they have divergent effects on the HPA axis. SD stress is a negative stressor (distress) and has been shown to induce HPA axis dysfunction, whereas physical exercise and enriched housing—both of which are positive stressors (eustress)-—render the HPA axis more adaptive. We hypothesized that the persistent arousal of HPA activity by SD contributes to the pathogenesis of depression. We also hypothesized that HPA activity during EE is requisite for the restoration of normal phenotype in depressed mice. Because the hippocampus provides inhibitory control on HPA axis output, and because hippocampal neurogenesis has been implicated in the pathology of depression, we also hypothesized that divergent outcomes of eustress and distress exposure are hippocampal neurogenesis-dependant. We designed three experiments to test these hypotheses.

Methods: We first examined if adrenalectomy (ADX) coupled with basal corticosterone (CORT) replacement would alter how animals respond to SD. We exposed ADX and sham-operated male C57BL6/J mice to 14 days of chronic SD and measured the expression of maladaptive behaviors. We also examined the survival of newborn hippocampal neurons during SD. Next, we examined the involvement of adrenal glucocorticoids in the restorative effects of environmental enrichment (EE) in defeated mice. We hypothesized that HPA activity during EE are required for behavioral recovery after defeat stress. Male mice were exposed to SD for 2 weeks then either ADX (and basal CORT-replaced) or sham operated. Mice were then housed in EE for 3 weeks and subsequently examined for affective behavioral disorders. Lastly, we tested if decreased neurogenesis contributes to the etiology of SD-induced depression or if it is downstream from changes in behavior. We hypothesized that SD results in depressive-like behavior through a glucocorticoid-induced decline in surviving hippocampal neurons and the protective effects of ADX in distress would not persist in mice with conditionally suppressed neurogenesis (NG-). Suppression is achieved through the administration of ganciclovir to the diet of GFAP-HSVtk transgenic mice. NG- mice

and their wildtype (WT) littermate controls were ADX and exposed to defeat stress as described in the first experiment.
Results: Mice ADX prior to social defeat showed decreased anxiety- and depressive-like behaviors and increased survival of adult born hippocampal neurons compared to sham-operated mice. During behavioral recovery after SD, sham-operated mice housed in EE showed decreased expression of anxiety- and depressive-like behaviors and increased survival of adult born neurons. However, if we ADX defeated mice prior to EE, the beneficial effects of EE disappeared, and hippocampal neurogenesis was decreased. Lastly, while ADX conferred stress resiliency to the WT mice, ADX NG- mice lacked resiliency and developed depressive behavior.
Discussion: The current experiments show a strong correlation between survival of adult-born hippocampal neurons, adrenal corticosteroids, and expressions of affective disorders. For instance, HPA axis stimulation during EE promotes behavioral recovery in defeated mice and ADX prevents behavioral depression from social defeat. Both manipulations promote the survival of adult-born hippocampal neurons; HPA-activity during EE increases survival and ADX before SD protects against decreased survival. Decreased hippocampal neurogenesis and dysregulation of the HPA axis are implicated in the etiology of depression. The finding of the present study—demonstrating that ADX protects WT mice from the detrimental effects of SD but did not protect mice with conditionally suppressed neurogenesis (NG-)— suggests that SD causes depressive behavior through CORT-induced decreases in hippocampal neurogenesis. Because ADX did not afford protection from defeat in NG- mice, this suggests that decreased hippocampal neurogenesis—even in the absence of high-levels of CORT—is sufficient to eliminate stress-resilience in mice.
Disclosure: M. Lehmann: None. **R. Brachman:** None. **K. Martinowich:** None. **R. Schloesser:** None. **M. Herkenham:** None.

160. Supraphysiological Levothyroxine Treatment improves Mood in Association with Limbic Metabolism of Bipolar Depressed Patients: A Randomized, Placebo-Controlled Study

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Background: Thyroid hormones play a critical role by modulating metabolic activity both in the developing and the adult brain. Using positron emission tomography (PET) with [(18)F]fluorodeoxyglucose (FDG), we previously showed in a study of bipolar depressed patients that cerebral metabolism decreased after 7-week open-label treatment with supraphysiological doses of levothyroxine (L-T4) in a network of limbic structures (striatum, amygdala, hippocampus, thalamus, subgenual cingulate, cerebellar vermis), with the reductions within amygdala, hippocampus and striatum significantly correlated with clinical gains (reduced HAMD scores) (Bauer *et al*., 2005). We now report on findings from a new sample of 25 patients who received levothyroxine or placebo treatment in a randomized, controlled trial.
Methods: This was a 6-week, double-blind, randomized, placebo-controlled study assessing efficacy of L-T4 (300 mcg/d, fixed dose) adjunctive to continuing treatment with mood stabilizer and/or antidepressant medication for patients with bipolar disorder currently depressed. Further inclusion criteria: either sex, age 18 years or older, normal thyroid (TSH) status, stable dosage of mood stabilizer and/or antidepressant for at least 2 weeks before randomization, serum levels of mood stabilizer within therapeutic ranges for at least 2 weeks, and antidepressants at standard doses. Exclusion criteria: ultra-rapid cyclers and thyroid hormone medication within 4 weeks before screening. The primary clinical measure of depression was change in HAMD total score from randomization to completion of treatment. Regional brain activity was assessed with PET and [^{18}F]-FDG in patients before (Scan 1) and after (Scan 2) treatment. The primary biological measures were relative regional activity (with relative brain radioactivity taken as a surrogate index of glucose metabolism) in pre-selected (from our previous study, see above and reference) brain regions. Treatment-associated changes in regional activity (relative to global activity) were tested against clinical response using statistical parametric mapping.
Results: Of the 25 patients who completed the PET study, 15 were randomized to receive L-T4, and 10 to receive placebo. Mean HAMD score at the time of randomization was 21.7 ± 3.2 in the L-T4 group, 20.3 ± 6.3 in the placebo group. Although 30% of placebo-treated patients showed markedly reduced HAMD scores from randomization to week 6 (>50%), the mean decrease, measured via one-tailed t-test, was significantly larger in the L-T4 group (-9.3 (42.8%) vs. -4.0 (19.7%) $p = 0.031$). L-T4 treatment was associated with decreased brain activity in all expected structures post-treatment (p's = 0.019 to 0.001), and the decrease was correlated with the decrease in HAMD score (p's = 0.019 to 0.0005). Relative brain activity did not decrease significantly in any structure, nor was it correlated with HAMD score, after placebo treatment (all $p > 0.05$). Both metabolic decrease and its correlation with HAMD score were significant, however, across the two groups in all structures ($p < 0.05$). Moreover, there were no significant Scan x Group interactions, and a significant HAMD Score x Group interaction only in the thalamus ($p = 0.024$).
Discussion: This is the first placebo-controlled study to evaluate clinical efficacy and brain metabolic effects of treating bipolar depressed patients with supraphysiological doses of L-T4. We replicated the clinical improvement, the decrease in limbic metabolism, and the correlation of these two effects after L-T4 treatment, observed in a prior study without placebo treatment. Although the L-T4 and placebo groups differed in clinical response, they did not differ significantly in metabolic response to treatment, and only the thalamus showed a difference between groups in the relationship of clinical response to metabolism. Coupled with the marked clinical improvement in 30% of placebo patients and the correlation of metabolic with clinical response across all patients, these results suggest that placebo-mediated mood improvement produces metabolic changes in the limbic system similar to those associated with levothyroxine-mediated mood improvement.
Ref: Bauer M *et al*. (2005) Supraphysiological doses of levothyroxine alter regional cerebral metabolism and improve mood in women with bipolar depression. Mol Psychiatry 10:456-469.
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Disclosure: M. Bauer: Part 1: Member of advisory board for AstraZeneca, Lilly, Servier, Lundbeck, Bristol-Myers Squibb and Otsuka. Speaker Honoraria from AstraZeneca, Lilly, Lundbeck, GlaxoSmithKline, Bristol-Myers Squibb and Otsuka., Part 2: from my employer: University Hospital Carl Gustav Carus Dresden, Dresden, Germany, Part 3: none, Part 4: from my employer: University Hospital Carl Gustav Carus Dresden, Dresden, Germany. **S. Berman:** None. **T. Stamm:** Part 1: Speaker Honoraria from AstraZeneca. **M. Adli:** Part 1: Advisory board: Bristol-Myers Squibb, esparma, and Lundbeck. Speaker honoraria:Astra Zeneca, Eli Lilly & Company, Lundbeck, Bristol-Myers Squibb, GlaxoSmithKline, Pfizer, Boehringer Ingelheim, Sanofi, esparma, Wyeth Pharmaceuticals, and Deutsche Bank., Part 4: Grant support from German Federal Ministry of Education and Research, German Federal Ministry of Health, the Volkswagen-Foundation, Lundbeck, Bristol-Myers Squibb. **M. Pilhatsch:** None. **E. London:** None.

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161. Avoidance, Safety Behaviour, and Reassurance Seeking in Generalized Anxiety Disorder

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Background: In contrast to other anxiety disorders, the behavioural symptoms of Generalized Anxiety Disorder (GAD) are not well characterized. The present study examines the behavioural symptoms in patients with GAD compared to healthy controls without psychopathology as well as their change during therapy and their predictive value for short- and longterm outcome.
Methods: Secondary data analysis of N = 56 patients with DSM-IV GAD from a randomized controlled trial testing worry exposure (n = 29) and applied relaxation (n = 27), compared to N = 33 demographically matched healthy controls without psychopathology. Participants reported on cognitive and behavioural avoidance, safety behaviour, and reassurance along with reports on physical symptoms and severity/impairment. The Hamilton Anxiety Scale and the Penn State Worry Questionnaire served as immediate and long-term treatment outcome measures. Regression analyses were conducted for between- and within-group comparisons.
Results: GAD patients engage significantly more in cognitive and behavioural avoidance, safety behaviour, and reassurance seeking than healthy controls. Treatment with worry exposure or applied relaxation reduces these behavioural strategies significantly without substantial indication of differential effects. However, only patients remitting from GAD reach the low level of healthy controls on the range of behavioural symptom measures. The initial level of behavioural symptoms in GAD is largely irrelevant for treatment success, but higher degrees of cognitive and behavioural avoidance as well as safety behaviour at the end of treatment predict worse long-term outcome.
Discussion: Behavioural symptoms are relevant components in GAD that improve with successful treatment. Inclusion of behavioural symptoms in the definition of GAD may be warranted in future diagnostic schemes to facilitate recognition and guide treatment.
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162. Pilot Study of Mindfulness-based Exposure Therapy for PTSD in OEF/OIF Veterans: Preliminary Clinical Outcomes and Pre-post fMRI Neuroimaging

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Background: Combat PTSD is a debilitating disorder affecting up to 20% of returning veterans. Trauma-focused therapies such as prolonged exposure (PE) show proven efficacy, but additional approaches are needed especially for patients who will not engage in PE. Mindfulness-based Cognitive Therapy (MBCT) shows substantial efficacy for prevention of depression relapse, and our pilot study of MBCT adapted for PTSD also found good acceptability and a significant reduction in PTSD symptoms (~25%) in combat veterans with PTSD. Here we report initial findings of pilot groups with a novel manualized 16-week mindfulness-based, PTSD-specific group intervention that combines mindfulness training, self-compassion exercises, and *in vivo* exposure - "Mindfulness-based Exposure Therapy" (MBET). Pre and post fMRI data suggest potential neural correlates of associated therapeutic change.
Methods: A total of n = 22 combat veterans of OEF (Afghanistan) and/or OIF (Iraq) seeking treatment for PTSD at the VA Ann Arbor were sequentially recruited for pilot groups: two MBET (n = 15) groups and one group of a manualized comparison therapy, "Present-Centered Group Therapy" (PCGT) previously developed as a comparison therapy for trauma-focused therapies (n = 7). Psychiatric interviews with an independent assessor (CAPS and MINI) and self-report measures were obtained at intake (pre), 8 weeks (mid), 16 weeks (post-therapy), and 28 weeks (3 mo FU) . Emotional regulation fMRI neuroimaging was conducted in a 3T environment, using emotional probe (IAPS pictures) and emotional regulation tasks (re-appraisal and meta-cognitive "rating" tasks). Scans were performed before the start of therapy, and post-therapy following assessment.
Results: Drop rate was high in both groups, as often seen in PTSD intervention studies. In MBET completers, acceptability and compliance both in session and homework appeared good. Age, combat exposures, and PTSD symptoms were not different at intake in completers. CAPS scores improved significantly in MBET completers (mean decrease 25.7 ± 10.1, $t[8] = 2.6$, $p = .03$, Hedges $g = 1.2$) but not in the PCGT completers (mean decrease 9.7 ± 7.8, $t[3] = 1.2$, $p = .38$). Intent-to-treat (ITT) analysis of the n = 12 subjects who began MBET also found a significant reduction in total CAPS (average decrease 19.7 ± 8.1, $t[11] = 2.1$, $p = .03$). MBET also showed Improvement in self report measures of PTSD, rumination, anxiety and depression, and "non-reactivity" facet of mindfulness (FFMQ). fMRI data are currently available from n = 11 patients pre-therapy, and n = 8 post-therapy. Pre-therapy, patients showed activation of bilateral amygdala, visual cortex, and IFG in viewing aversive IAPS, and greater activation of dorsal ACC (10, 48, 20, Z = 3.0) and right IFG (33, 27, -6, Z = 3.2) in "rating" condition. Preliminary analyses of pre-post scans (MBET n = 4, PCGT n = 4) found reduced activation of amygdala (-18, -6, -15, Z = 3.5) post-MBET while viewing aversive IAPS, and a factorial Group x Time interaction in peri-amygdala (-27, -3, -21, Z = 3.2) and dorsal ACC (18, 30, 27, Z = 4.4).
Discussion: Our preliminary data analysis suggests that MBET group therapy for PTSD appears well-tolerated by OEF/OIF combat veterans seeking treatment, and may lead to clinically meaningful improvement in PTSD symptoms. While the improvement in PTSD symptoms in MBET was not as large as reported for individual trauma-focused therapy (e.g. PE), it compares favorably with other published reports of group therapy for PTSD. fMRI neuroimaging results are highly preliminary, but suggest possible changes in emotional neurocircuitry that may accompany clinical change. A randomized controlled trial of MBET compared to PCGT, with pre-post fMRI, is currently underway.
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163. Clinical Survey of the Mother-Infant Mental Health Clinic: Psychiatric Characteristics of Consecutive 109 Patients

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Background: Optimal care and treatment strategy should be organized for each mother throughout pregnancy and postnatal period at best timing. The Mother-Infant mental health clinic in Kyusyu University Hospital provides pregnant women with special psychiatric care since 2002. The aim of the study is to analyze clinical features of these women who received care and to propose our treatment strategy. Clinical services have

been provided by multidiciplinaryteam in the hospital such as obstetric, psychiatric, pediatric staffs. These services are also collaborate with health visitors and social workers in the community.
Methods: 1) Recruitment: Women with mental health disorders currently or in the past or, with psychosocial problems such as lack of emotional support are recommended to visit our clinic. The recruitment was carried out from 2002 to 2009. Mothers to be recruited are firstly, patients with present psychiatric disorders, secondly, patients with past psychiatric disorders and thirdly patients with poor emotional or social support. One hundred nine pregnant women were recruited between two thousand two to two thousand nine. 2) Instruments and procedures: At third trimester, Structured Clinical Interview for DSM-(SCID) was performed. The Edinburgh Postnatal Depression Scale (EPDS, Cox, 1987) was performed at 5 days, one month, four months and seven months postnatally. At seven months, infants' developmental outcome was assessed using the Denver Developmental Screening test at seven months postnatally.
Results: Patients are categorized as having mood disorders (n = 29) anxiety disorders (n = 27), schizophrenia (n = 10), other psychiatric diagnosis (n = 21), no psychiatric disorders (n = 22) with many of them are socio-economic problems with poor social or emotionalsupport . In recent years those with psychosocial adversity are increasing . EPDS marked over cut-off point during pregnancy and also after birth, in these mood disorder group and anxiety disorder group. Infants of the mothers with mood disorder and anxiety disorder are likely to be born with full term and within normal birth weight range, They were fborn at 39.0 and 38.6 gestational weeks respectively, and birth weight were 3078 gram and 3040 gram). Infants of the mothers with schizophrenia are likely to be born earlier (38.0 gestational weeks) have lower birth weight (2789gram). One developmental disorder is observed in both offspring of mood disorder and schizophrenia. Regarded pharmacotherapy in pregnancy, no fetal risk was observed among our cases. For example, in our closed monitored seven cases with SSRI therapies during pregnancy, none of the infants was born prematurely, the birth weights were over two thousand five hundred grams, and at seven months all of the infants developed normally.Although longitudinal follow up in larger sample should be needed, sufficient amount of drug therapy might be approved considering risk-benefit balance.
Discussion: Preliminary data of Mother-Infant mental health clinic was presented. The impact of mood or anxiety disorders on infant development was not obvious, however a little concern should be paid for the infants of schizophrenic mothers due to their lower birth weight and possible risk of suffering from poor parenting from their mothers.
Disclosure: K. Yoshida: None. **Y. Fujinaga:** None. **H. Yamashita:** None.

164. EEG Predictors of Response to Brain Computer Interface Treatment in ADHD

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Background: Attention-deficit hyperactivity disorder (ADHD) is a major childhood psychiatric disorder. We have developed a novel Brain-Computer Interface (BCI) system for the treatment of ADHD. Using dry electrode sensors on the forehead (FP1 and FP2), EEG signals can be extracted and analyzed from subjects. These signals are enhanced by a spatial filter to differentiate attentive and non-attentive states. The attention information derived from the subject is used to drive a computer game in an asynchronous feed-forward mode. We devised a training protocol comprising of a series of computer games and homework tasks. Here we investigate EEG predictors of response, longitudinal treatment effects and the correlation of EEG results with ADHD rating scale (ARS) scores.
Methods: This is a prospective, single arm, open-label, pilot study. Twenty children aged 6 to 12, diagnosed with ADHD (combined or inattentive subtype) based on DSM IV were recruited. These children were medication naïve and referred for the study as their parents declined medication. The study was approved by the ethics review board of the National Healthcare Group, Singapore, and registered with www.clinicaltrials.gov (NCT01344044). Participants underwent 24 individualized BCI intervention sessions over 2 months (3 sessions per week), followed by 3 months of once-monthly booster sessions, according to a manualized protocol. Each session lasted 30 minutes and involved playing a computer game that is driven by EEG-derived attention score (BCI score). Final assessment was at 6 months. Parents completed the ARS-IV at baseline, 2, 5 and 6 months. The BCI score, a proxy for the subject's attention level is built from the EEG data collected in the calibration session. During calibration, subjects complete a color Stroop task interspersed with resting conditions. These tasks represent attention and non-attention states of the subject. This parametric model consists of a filter bank array and a linear regression. The filter banks decompose EEG into a continuous array of frequency sub-bands that cover the range from 4 to 36 Hz. Machine learning techniques are used to derive linear regression mapping from the band powers into the BCI score. This model is built in a subject-dependent manner to capture specific EEG characteristics for each individual.
Results: Twenty subjects, 16 boys and 4 girls, with mean age 7.80 (SD 1.40) were recruited for this study. Fourteen were diagnosed with the combined, and six with the inattentive subtype of ADHD. Intention to Treat (ITT) analysis included 19 participants who did not drop out before 1 month. At 2 months as compared to baseline, the mean change (SD) in the parent-rated inattentive score on the ARS was -4.6 (5.9), $p = 0.004$, and -4.7 (5.6), $p = 0.001$ for the hyperactive score. When considering long-term effects, statistically significant changes ($p < 0.05$) at 6 month from baseline were found for both ARS scores. Mean changes (SD) were -5.0 (5.8), and -5.7 (5.1) for inattentive and hyperactive scores respectively.
Linear regression analysis showed that baseline BCI score significantly predicted the change in inattentive ARS sub-scores [beta(Se) = 0.81(0.04), $p = 0.04$]. Baseline BCI scores explained a significant proportion of variance in inattentive ARS sub-score changes ($R^2 = 0.26$, $F = 5.3$, $p = 0.04$). The change in BCI score significantly predicted the change in ARS scores [beta(Se) = -0.23(0.09), $p = 0.02$]. The BCI score change also explained a significant proportion of variance in ARS score changes ($R^2 = 0.37$, $F = 6.9$, $p = 0.02$).
Discussion: There was significant improvement in the ARS scores post-treatment and they were sustained over six months with only monthly booster sessions. We postulate a possible enduring learning effect. We found that changes in EEG-derived BCI score correlates with changes in ARS scores.This suggests that the BCI score may be an indicator of ADHD symptom severity. Moreover, baseline BCI score appears to predict the change in inattentive ARS sub-scores after treatment. A subject who starts off with a lower baseline BCI score ('poorer attention') improves more in terms of inattentive symptoms. Hence the BCI score at baseline can potentially be a predictor of treatment outcome. The limitation of this pilot study is that it is single arm and open label, with a small sample size. A larger double blinded randomized control trial with a longer follow-up period is underway to further assess the clinical efficacy and investigate the role of the BCI-score as a proxy of attention and predictor of treatment response.

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165. $GABA_A$ and TLR4 Innate Immunity Receptors of the Ventral Tegmental Area Regulate Nicotine Sensitization in Alcoholic Rats

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Background: Tobacco use and heavy alcohol consumption represent a significant public health concern. Thus, studies that investigate the molecular underpinnings of sensitivity to nicotine in alcoholic subjects may contribute substantially to our understanding of treatments for comorbid dependence. In the present study, we tested the hypothesis that the α1- and α2-containing $GABA_A$ receptor subunits and the innate immunity receptor [TLR4], which was recently demonstrated to play a significant role in regulating excessive alcohol drinking, might be salient in regulation of nicotine-induced sensitization in alcohol-preferring [P] rats. The neural adaptations that occur in brain reward circuits following sensitization may model the development of compulsive drug use.
Methods: Employing the Kelley *et al.* sensitization paradigm (Schroeder *et al.*, 2001), P and NP rats were sensitized over a 15 – 18 day period using 90 min, open field, locomotor sessions. Subsequently, P rats were microinfused with siRNA amplicon vectors in the ventral tegmental area [VTA]. The amplicon vectors comprised the α1/α2 $GABA_A$ receptor subunits and the TLR4 innate immunity receptor (see Liu *et al.*, PNAS, 2011). VTA protein levels were confirmed using Western immunoblot assays before and after microinfusion. All experimental protocols were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*
Results: Relative to NP rats, P rats demonstrated an enhanced sensitivity to the locomotor-stimulating effects of nicotine; NP rats evidenced little if any stimulation by nicotine. Immunoblots revealed significantly higher levels of the α1, α2, and TLR4 receptors in P relative to NP rats in the VTA. In comparison with the scrambled amplicon, the siRNA α1, α2, and TLR4 amplicons markedly reduced locomotor behavior on Day 1. This reduction attenuated across a 10-day period, with animals reaching pre-viral infusion levels around Day 12. In parallel with behavioral changes, protein levels returned to approximate basal levels on Day 12.
Discussion: The data suggest that rats with a genetic predisposition to alcoholism evidence a markedly enhanced sensitivity to nicotine's locomotor-activating effects. It is possible that the innately overactive $GABA_A$ and TLR4 systems in the VTA may modulate these effects. The marked reductions observed with the siRNA α1, α2, and TLR4 amplicons lends creedence to our hypothesis. Given their neuroanatomical localization, it is further possible that $GABA_A$ and TLR4 innate immunity receptors modulate dopaminergic neurons in attenuating the effects of nicotine sensitization.
Disclosure: K. Warnock: None. **J. Liu:** None. **T. Kelly:** None. **L. Aurelian:** None. **H. June:** None.

166. Real-time fMRI Neurofeedback Targeting Inhibitory Control Brain Activation decreases Emotional Reactivity to Smoking Cues in Cigarette Smkers

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Background: Addiction is associated with dysfunction in two brain circuits involved in: (1) reward (e.g., striatum/nucleus accumbens and ventromedial prefrontal cortex, VMPFC) and (2) inhibitory control (e.g., dorsolateral prefrontal cortex, DLPFC and inferior frontal cortex, IFC). Cue-induced craving in smokers has been found to trigger relapse via these brain systems. Smokers have demonstrated that they can effectively use emotion regulation strategies to modulate these brain systems in response to visual smoking cues. Real-time functional magnetic resonance imaging (rtfMRI) neurofeedback is a promising new clinical tool to help people self-regulate regional brain activation and associated psychological processes by providing them with information about changes in their brain activation. The goal of this study was to establish that rtfMRI neuromodulation in combination with emotion regulation strategies is a feasible intervention option for decreasing emotional reactivity to smoking cues in cigarette smokers.
Methods: We collected fMRI data from 20 cigarette smokers who engaged in passive viewing of smoking-related visual cues to investigate brain activation in regions involved in reward (striatum/nucleus accumbens, VMPFC) and inhibitory control (DLPFC, IFC). We also used rTfMRI in conjunction with emotion regulation strategies to aid 5 smokers in learning to modulate activation in cue reactive reward and inhibitory control brain regions. Participants also rated smoking cues on their emotional value and reported their craving experience pre- and post-scanning session.
Results: Of the 17 participants with analyzable data, 14 exhibited robust activation in reward and inhibitory control-related brain regions in response to the smoking cues (Fig. 1). Five smokers completed 4 reward and 3 inhibitory control neuromodulation sessions.Three of 3 participants showed an increase in inhibitory control modulation.One of 4 participants showed a similar change in reward modulation. Overall, participants appeared to improve control of inhibitory control regulation following feedback training (Fig. 2). Interestingly, all participants reported a decrease in pleasantness (7/7 sessions) and/or desirability (6/7 sessions) of smoking cues following neuromodulation procedures (Fig. 3). The participant who demonstrated the greatest increase in inhibitory control modulation reported the greatest decrease in pleasantness and desirability of the smoking cues following neuromodulation and a decrease in the number of cigarettes smoked daily from 20 to 5 in the week following the second neuromodulation session.
Discussion: Overall, participants (1) showed improved control of inhibitory control > reward ROI regulation and (2) reported decreased emotional reactivity in response to smoking cues following rTfMRI training. These preliminary data support the possibility that smokers can learn to control smoking cue associated brain activation using emotion regulation strategies in conjunction with rtfMRI neurofeedback and that they may be able to modulate inhibitory control-related brain regions more effectively than those involved in reward processing.
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167. Preliminary Results Showing That Treatment Seeking Cigarette Smokers Can Use Realtime fMRI Feedback of Regional Brain Activity to Reduce Cue Induced Craving and Regional Blood Flow

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Background: Numerous research groups are now using analysis of blood-oxygen-level dependent (BOLD) fMRI results and

relaying information about regional activity to participants in the scanner in "real time". In this study we explored the feasibility of self-regulation of frontal cortical activation through real time functional magnetic resonance imaging (rtfMRI) biofeedback in nicotine-dependent cigarette smokers during exposure to smoking cues.
Methods: Ten cigarette smokers were shown smoking-related cues in a 3T MRI scanner to induce nicotine craving. Participants were instructed to modify their craving based on regional brain activation (rtfMRI feedback) using two different approaches. In a "reduce craving" paradigm, participants were instructed to 'reduce' their craving, and decrease the thermometer level, which reflected activity from the from the anterior cingulate (ACC). In a separate "resist" paradigm, participants were given feedback from a region within the middle prefrontal cortex (mPFC) and asked to increase their resistance to craving in the presence of smoking cues and to increase reading on a thermometer reflecting mPFC activity. They were asked to rate their craving under both circumstances. Online fMRI imaging data were analyzed with Turbo-Brain Voyager 2.0 (TVB) and offline imaging data were analyzed with Statistical Parametric Mapping software 8 (SPM 8).
Results: Participants were able to significantly reduce the BOLD signal in the ACC during the "reduce craving" task ($p = 0.028$). Their self-report craving ratings during exposure to smoking cues were lower during the 'reduce craving' biofeedback scan as compared to the baseline scan ($p = 0.002$). Additionally, there was a significant correlation between decreased ACC activation and reduced craving ratings during the "reduce craving" scan ($p = 0.011$). In contrast, there was no modulation of the BOLD signal in mPFC during the "increase resistance" scan and no significant difference in craving ratings between the resisting baseline scan and the "increase resistance" biofeedback scan.
Discussion: These preliminary results suggest biofeedback via rtfMRI can be used to voluntarily regulate ACC activation and reduce smoking cue-induced craving. In addition, it appears that individuals were more able to manipulate brain activity via biofeedback rtfMRI with the instruction to "reduce craving" with ACC feedback as compared to the "increase resistance" with mPFC feedback. The current study may be helpful in defining the optimal parameters for the study of biofeedback rtfMRI as a therapeutic tool for nicotine dependence.
Disclosure: M. George: Part 1: Research Grants: Brainsway, Mecta, Neuronetics, Neurosync, Neurostim Consulting: Puretech Ventures Purchased Research Equipment: Magventure, Part 4: Research Grants: Brainsway, Mecta, Neuronetics, Neurosync, Neurostim. **X. Li:** None. **K. Hartwell:** None. **J. Borckardt:** None. **J. Prisciandaro:** None. **M. Saladin:** None. **P. Morgan:** None. **K. Johnson:** None. **T. LeMatty:** None. **K. Brady:** None.

168. Cortical Excitability in Current Cocaine Users: A Transcranial Magnetic Stimulation Study Investigating Glutamatergic And GABAergic Processes

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Background: While the majority of the research on the effects of chronic cocaine on the brain has targeted subcortical areas, emerging evidence suggests that there are fundamental alterations in cortical excitability in cocaine users. Prior imaging and electrophysiological studies have demonstrated that current cocaine users have elevated cortical activity and a loss of typical cortical laterality when doing a basic motor task. It is unclear however if the elevated cortical activity observed in cocaine users is due to an increase in excitability or a decrease in inhibition.
Methods: In the present study, transcranial magnetic stimulation was used to assess multiple aspects of cortical tone in both the left and right hemisphere in a preliminary set of six current cocaine dependent users and six non-drug dependent controls matched for gender (50% female), age (36yo ± 2.4), education (13.5 yrs ± 1.0), current nicotine use, and current alcohol use. Users reported time of last use between 12-72 hours prior to the study which was verified with urinanalysis. The techniques used to investigate cortical tone included: paired pulse intracortical inhibition (ICI, 3 ms delay) and intracortical facilitation (ICF, 15 ms delay), cortical silent period (CSP), and the recruitment curve (RC).
Results: Consistent with prior studies, current cocaine users had significantly lower resting motor thresholds (RMT) and lower short interval cortical inhibition than controls in the left hemisphere. Although the motor threshold in the controls was higher in the right than the left hemisphere, there was no interhemispheric difference in the cocaine users. The cocaine users had consistently shorter cortical silent periods than the controls in the left and right motor cortex. There was no difference in cortical facilitation or the slope of the recruitment curve in either the left or right hemisphere of cocaine users.
Discussion: Considered together these data suggest that the elevated cortical excitability observed in the left and right motor cortex of cocaine users (RMT) is associated predominantly with GABAergic processes (SICI, CSP) rather than glutamatergic dysregulation (LICI, RC). Although this is a preliminary study, these data may provide insight into the relative value of GABAergic and glutamatergic agents as a treatment for these individuals. Given that GABAergic sensitivity is modulated by myelin, these data also support emerging literature on altered myelin integrity in cocaine users.
Disclosure: C. Hanlon: None. **S. Fredrich:** None. **M. George:** Part 1: Research Grants: Brainsway, Mecta, Neuronetics, Neurosync, Neurostim Consulting: Puretech Ventures Purchased Research Equipment: Magventure, Part 4: Brainsway, Mecta, Neuronetics, Neurosync, Neurostim Neuronetics.

169. Deep Brain Stimulation in Treatment Resistant Alcohol Addiction - Longterm Results of the Magdeburg Pilot Study

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Background: Worldwide, over 76 million people suffer from alcohol use disorders. They are at increased risk for early death by somatic complications with a reduced life expectancy by over 20 years and show an increased suicide risk compared to the nonpsychiatric ill. Unfortunately, following current treatment options, over 50% of alcoholics do not achieve long-term abstinence and subsequently develop severe complications. Aside from these individual medical consequences, addiction causes an immense financial damage to society. Thus, finding new treatment options remains a leading challenge in clinical psychiatry. In the last years, there has been increased evidence that chronic alcohol consumption leads to a dysfunction of the Nucleus accumbens (NAc), thereby causing cue-induced craving, which is considered one of the main reasons why patients cannot stay abstinent and relapse. The observation of remission of a secondary alcohol addiction in a patient who received deep brain stimulation of the NAc for treatment resistant anxiety disorder led to the initiation of this trial.
Methods: On the basis of an OFF-label single patient use, 5 patients with very severe treatment resistant alcohol addiction for many years received bilateral deep brain stimulation to the Nucleus accumbens at our centre. They were operated in general anaesthesia using a modified Riechert-Mundinger stereotactic system with a deep fronto-lateral approach. Quadrupolar electrodes (Medtronic Quad 3389) for DBS were placed bilaterally in the

shell region of the nucleus after presurgical planning using high resolution T1-weighted MRI-scans. Subsequently, electrode-cables were connected to an impulse generator located beneath the left pectoral muscle (Kinetra, Medtronic®) similar to a cardiac pacemaker.
Results: All patients reported an immediate and ongoing absence of craving for alcohol. All patients show a severe reduction of drinking days and amount of drinks/day. 4/5 patients are continuously employed again after years of unemployment. 2 patients remain completely abstinent for 4 years. Aside from 4 very short relapses for 2-3 days each a third patient remains abstinent as well.
Discussion: DBS of the NAc showed to be safe and effective in this small sample of 5 patients with a very severe treatment resistant alcohol addiction. As relapses to drinking are considered to be a part of disease in this subsample of severely ill patients, hard-reduction strategies such as reducing the days drinking and drinks per day are increasingly being considered as a primary treatment option. Therefore, our results even exceed this goal clearly in 3 out of 5 patients, whereas the other patients still fulfil these criteria. Based on this favourable pilot data, we believe that a randomized and sham-controlled clinical trial is justifiable and required to further study the possibility of DBS as a novel treatment option in alcohol addiction. The prospective, randomized, double-blinded and sham-controlled multicenter study DeBraSTRA (Deep Brain Stimulation in Treatment Resistant Alcoholism) has been authorized by federal authorities in Germany and will be funded entirely independent from industries by the German Research Foundation.
Disclosure: U. Mueller: None. **B. Bogerts:** None.

170. Plasma Levels of Vasopressin are Altered in Response to Opioid Receptor Modulation: Mass Spectrometric Quantification

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Background: The detection of peptide hormones in plasma is central to the clinical and laboratory study of neuroendocrinology. The means for quantification of peptide hormones present at subnanomolar levels in plasma rely on antibody based techniques, especially radioimmunoassay (RIA) and enzyme-linked immunosorbent assays. As a quantification means, antibody based techniques are generally sensitive, but lack absolute specificity, with the potential for cross-reactivity with alternative forms of the targeted analyte, including degradation products and post-translational modifications. Mass spectrometry has the potential to offer unambiguous detection, as well as the potential for general detection of related products which antibody based methods may not be able to distinguish. Of the known peptide hormones, upwards of 100 different entities with amino acid chain lengths of 2-100 amino acid residues, vasopressin has been particularly well studied, in part due to its relatively early discovery and structural elucidation, and in part due to chemical properties which allows for particularly good antigenicity. The ability to quantify vasopressin in plasma using RIA has allowed rigorous study of its peripheral regulation in response to both endogenous physiological phenomena as well as various pharmacological exposures, which thus makes it an ideal target for initial development of spectrometric methods for the quantification of peptide hormones present at very low concentrations in plasma. We initially developed targeted multistage (ms^3) mass spectrometry for the detection of the peptide hormones vasopressin and oxytocin in plasma, the first demonstration of this technique for the detection of endogenous peptidic hormones. The current work extends these developments to absolute quantification of vasopressin in plasma, with cross-validation of results from studies utilizing RIA demonstrating the responsiveness of peripheral vasopressin to opioid receptor activation.
Methods: Sprague Dawley rats, maintained under stress-free housing conditions, were injected with 8 mg/kg morphine and sacrificed 4 hours later via decapitation under isoflurane anesthesia. Trunk blood was collected, followed by plasma collection. Stable isotope labeled standards were developed and added for quantification purposes. For the plasma levels of vasopressin, we add 2.5 pM of single Dalton standard to the plasma prior to further preparation. The plasma was acidified, and subject to ultrafiltration, followed manually loaded onto a 75 µm i.d. column, for subsequent HPLC-mass spectrometry. A LTQ ion trap mass spectrometer (Thermo) was utilized for multistage ms^3mass spectrometric detection and quantification.
Results: In the plasma of Sprague-Dawley rats, the baseline levels of vasopressin were found to be 2.2 pM. Treatment with 8 mg/kg morphine led to a small increase in vasopressin levels (3.3 pM), as expected. The use of different types of stable isotope labels were compared methodologically to determine the best methods for quantification in ms^3 mode.
Discussion: Studies using RIA previously demonstrated the presence of vasopressin in the plasma and responsiveness to opioid agonists. 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 vasopressin in rat plasma, with validation compared to previous results using radioimmunoassay, thus *demonstrating for the first time the use of mass spectrometry for the quantification of a peptidic hormone in plasma present at subnanomolar concentrations*. Our innovative methodology will potentially transform the manner in which investigations of the endocrinological and neurochemical underpinnings of many disorders are conducted.
Disclosure: B. Reed: None. **B. Chait:** None. **M. Kreek:** None.

171. Experienced Oioid Abusers' attempts to Prepare a Crush-Resistant Oxymorphone Extended-Release Formulation for Intranasal or Intravenous Use

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Background: When taken as prescribed, prescription opioid medications are effective for pain management, yet national statistics reveal high levels of nonmedical use. Common methods of abuse include crushing the pills to create a powder for insufflation (snorting) or creating a solution for intravenous use (shooting); these techniques are typically employed to circumvent extended-release (ER) mechanisms and induce a potent opioid high. Tamper resistant formulations of prescription opioids have been developed to deter abuse by these methods. Oxymorphone hydrochloride ER (Opana® ER) tablets have been available since 2006 for the treatment of pain.The purpose of the present studies was to examine whether experienced prescription opioid abusers were able to prepare a crush-resistant formulation (CRF) of oxymorphone hydrochloride ER 40 mg for intranasal (Study 1) or intravenous abuse (Study 2), utilizing an Opana® ER 40 mg tablet (OPN) as a positive control.
Methods: *Study 1.*Participants were provided with the CRF and OPN tablets in random order and asked to prepare them for intranasal use with tools /solutions that they had requested previously. No drug was ingested. The primary outcome measure was particle size distribution. Secondary endpoints were time spent preparing the tablets, maximum time willing to spend preparing the tablets, willingness to snort the tampered product,

and relative amount participants would be willing to pay for the tablets. *Study 2.* The same procedures as Study 1 were employed, except participants were asked to prepare the tablets for intravenous use. The primary outcome was percent yield of active drug extracted as solution. Secondary outcomes were time spent preparing the tablets, maximum time willing to spend preparing the tablets, willingness to inject the tampering product, and relative amount participants would be willing to pay for the tablets.
Results: *Study 1.*Twenty-five predominantly black (52%) male (68%) participants with a mean (SD) age of 44 (11) years, who had been abusing prescription opioids for 8 (10) years, completed this study. The 3 most commonly used tools for intranasal preparation included hammers (92%), razors (56%), and wax paper (36%). By weight, fewer CRF than OPN particles were smaller than 1.705 mm (5.1% vs. 97.7%, $p < .001$). Participants spent more time preparing the CRF than the OPN for abuse (6 min vs. 3 min, $p = .001$). Maximum time participants were willing to spend preparing the tablets for abuse did not differ (10 min, CRF vs. 6 min, OPN). Almost all participants (96%) were able to render the OPN into a powder they were willing to snort, compared to 8% who were able to do so with the CRF ($p = .001$). Participants indicated they would pay "Less" (72%), or "Nothing" (28%) for the CRF when compared to the OPN. *Study 2.* Twenty-five predominantly white (64%), male (68%) participants with a mean (SD) age of 44 (8) years, who had been abusing prescription opioids for 15 (11) years completed this study. The 4 most common tools that were used for intravenous preparation included razors (80%), water (80%), spoons (72%), and lighters (68%). The percent yield of active drug solution did not differ between the CRF and the OPN (1.89% vs. 1.35%, respectively). Time spent preparing the solution for abuse did not differ (8 min, CRF vs. 9 min, OPN), nor did maximum time participants would be willing to spend preparing the tablets for abuse (16 min, CRF vs. 14 min, OPN). Few participants were willing to inject the solutions from the CRF (20%), or the OPN (25%), *not significant*. Most participants indicated they would pay ″Nothing″ (48%) or ″Less″ (32%) for the CRF than the OPN, with a smaller proportion indicating they would pay ″More″ (16%) or ″The same″ (4%) for the CRF as the OPN.
Discussion: Data from Study 1suggest that the oxymorphone hydrochloride CRF 40 mg reduced the ability of intranasal users to quickly and successfully prepare this medication for insufflation when compared to the Opana® ER 40 mg tablet. With regard to the preparation for intravenous use (Study 2), there were no differences in outcome measures between the oxymorphone hydrochloride CRF 40 mg and the Opana® ER 40 mg tablet, most notably in the small amount of active drug that was extracted, and in the few participants who were willing to inject either product. Thus, with regard to intravenous use, these data suggest that the CRF does not enhance the already-existing deterrent properties of the current ER formulation. Participants in both studies found less value in the CRF than the OPN formulation. Taken together, these data suggest that the crush-resistant formulations may deter abuse of Opana® ER 40 mg.
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172. Risperidone Long-acting Injections - How to Optimize the Treatment?

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Background: Risperidone long acting injection (RLAI) is the first injectable atypical antipsychotic combining advantages of an oral atypical antipsychotic with advantages of a depot formulation. The efficacy and safety of RLAI has been demonstrated in clinical trials,however, there is little information about the pattern and optimization of its usage in the real clinical practice. The aim: to assess RLAI usage patterns in patients participating in e-STAR (electronic Schizophrenia Treatment Adherence Registry) in both Czech and Slovak Republics.
Methods: e-STAR was designed to evaluate clinical outcome in patients who had initiated RLAI as a part of their continuing therapy in the routine clinical practice. The decision to initiate patients on RLAI and their clinical management were determined solely by the treating physician. The clinical and demographic data were collected at baseline and then prospectively every 3 months for 2 years. The clinical parameters included retention in the study, reasons for discontinuation, severity of illness according to CGI-S (Clinical Global Impression-Severity), global functioning (GAF - Global Assessment of Functioning Scale), social performance (PSP - Personal and Social Performance Scale), remission achievement and number of rehospitalizations. We focused on RLAI usage pattern (dosage, titration, concomitant medication).
Results: Totally 1 308patients were included (44% female, 56% male) with the diagnosis of schizophrenia 87%, with schizoaffective disorder 13% of treated, 89% of patients started the treatment in outpatient departments. The mean age was 38 years, mean duration of illness 9 years, only 10% were fully employed. The retention in the study was 82%. A significant decrease in the CGI- S and a significant increase in the GAF PSP scores was observed $p < 0.001$). The percentage of fully employed was the same. One fifth of the patients were minimally once hospitalized during the 2-year follow-up and one fifth achieved remission. The most frequent reason for change to RLAI was nonadherence and inefficacy. The initial RLAI dose was 25 mg every second week in 78% of the treated and there was no change of the dose during the study in a half of the patients. Half of the treated who discontinued for inefficacy had the low dose (25 mg). The mean dose at the study initiation was 28 mg at the study entry and 35 at the end of the study after 2 years. There was a significant decrease of coadministration of other psychopharmacs (statistically significant decrease of co-administered antipsychotics and anticholinergics ($p < 0.001$).
Discussion: In our sample RLAIs were given mostly after a long-term duration of illness in relatively low doses without titration. In spite of this a significant decrease of concomitant medication was observed. Further, a significant improvement of patientś functioning was seen and the occupational status did not change. The present observational multi-centre study suggests that we should try to find an optimal dose to improve the outcome and decrease polypharmacy. Further, we should use RLAI in the early phase of disease where there is a better chance to preserve employment.
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173. Gradual and Overlapping Antipsychotic Switch Strategies Are Associated with Less All-Cause Discontinuation in Schizophrenia: Results from a Meta-analysis of Different Switch Strategies

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Background: Antipsychotic switching is common in schizophrenia. However, randomized, controlled trials (RCTs) of different switch strategies have been scarce and inconsistent.

Methods: Meta-analysis of RCTs comparing different switch strategies in schizophrenia, defined by abrupt vs. slow initiation or discontinuation of the pre-switch or post-switch antipsychotic and by type of overlap (absent, partial or full = "plateau"). Primary outcome was all-cause discontinuation; secondary outcomes included specific-cause discontinuation, psychopathology and adverse effects. Pooled relative risk (RR) [+/-95%CIs] was calculated using random-effects model, with numbers-needed-to-treat/harm (NNT/NNH) calculations where appropriate.
Results: In eight trials, 1162 patients were randomized to 2 or 3 of 5 different switch strategies. Significantly more patients discontinued treatment with a) abrupt vs. gradual *initiation* of the post-switch antipsychotic (N = 2, n = 467, RR:2.49 (CI:1.43,4.35), p = 0.001; NNH = 14 (CI:9-33); b) abrupt vs. gradual *discontinuation* of the pre-switch antipsychotic (N = 7, n = 1109, RR:1.28 (CI:1.08,1.51), p = 0.004; NNH = 16 (CI:9-100), and c) non-plateau vs. plateau-switching (N = 5, n = 587, RR:1.42 (CI:1.12,1.79), p = 0.003; NNH = 11 (CI:7-25). Non-overlapping vs. partially/fully overlapping switching lead to significantly greater dropout when switching from atypical to atypical antipsychotics (N = 4, n = 683, p = 0.03), but not when switching from typical to atypical antipsychotic (N = 4, n = 426, p = 0.39). Global psychopathology changes did not differ across different switch strategies (p = 0.49-0.98), but few data were meta-analyzable (N = 1-3, n = 52-257). Abrupt vs. gradual discontinuation was associated with more insomnia (RR:2.61 (CI:1.07, 6.41; NNH 10 (CI:6-100), while abrupt vs. gradual initiation was associated with less akathisia (RR:0.61 (CI:0.39, 0.96); NNH = -13 (CI:-7 to -100).
Discussion: Non-abrupt and plateau antipsychotic switching leads to significantly lower all-cause discontinuation, especially during atypical antipsychotic switching.
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174. Long-Term Safety and Effectiveness of Lurasidone in Sshizophrenia: Results of a 22-Month, Open-Label Extension Study

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Background: This was a multiregional study designed to evaluate the safety, tolerability, and effectiveness of lurasidone in the long-term treatment of schizophrenia.
Methods: Subjects who successfully completed a 6 week double-blind (DB), placebo-controlled trial were eligible to continue in a 22 month open-label extension (OLE) study during which they received once-daily, flexible-dose treatment with lurasidone in the range of 40-120 mg. Safety and tolerability measures included adverse events (AEs), extrapyramidal symptoms, body weight, lipid parameters, prolactin, and ECGs. Efficacy assessments included the Positive and Negative Syndrome Scale (PANSS) total score, the Clinical Global Impression, Severity scale (CGI-S), and the Montgomery-Asberg Depression Rating Scale (MADRS).
Results: The proportion of subjects who discontinued was 48.6% at Month 6, 63.3% at Month 12, 69.7% at Month 18, and 73.3% at Month 24. Thirty-seven subjects (14.7%) discontinued due to an AE during the course of OLE treatment. Three AEs occurred with an incidence ≥10%: schizophrenia (12.4%), akathisia (10.8%) and somnolence (10.8%). Forty-eight subjects (19.2%) reported experiencing at least one movement disorder-related AE. The mean daily dose of lurasidone was 87.8 mg. At the original DB baseline, mean weight was 76.1 kg. Treatment with lurasidone was associated with a mean change from DB acute phase baseline (based on an observed case [OC] analysis) in weight of + 0.9 kg at Month 12, and + 0.7 kg at Month 24. Median change from acute phase baseline to Month 12 and Month 24, respectively, was -1.0 and -9.0 mg/dL for total cholesterol; 0.0 and -1.0 mg/dL for LDL cholesterol; + 1.0 and -11.0 mg/dL for triglycerides; + 4.0 and + 2.0 mg/dL for glucose; 0.0 and + 0.1 % for whole blood HbA1c; and -1.3 and -1.1 ng/mL for prolactin. During the course of OLE treatment, 2 subjects had a ≥60 msec increase in QTcF, and no subject had a QTcF interval >500 msec. There were no clinically meaningful changes in vital signs. The mean PANSS total score, for all subjects who continued into the OLE study (N = 250), decreased from 96.5 at the original DB baseline to 69.5 at OLE baseline. During OLE treatment, mean (95%-CI) change in the PANSS total score (OC analysis from OLE baseline) was -8.0 (-11.3, -4.7; n = 102) at Month 12, and -12.3 (-16.2, -8.4; n = 69) at Month 24. Mean (95%-CI) change in CGI-S was -2.0 (-2.2, -1.8) at Month 12 and -2.2 (-2.4, -2.0) at Month 24, based on OC analysis from DB baseline. Mean change in MADRS was -6.0 (-7.3, -4.7) at Month 12 and -6.5 (-8.2, -4.9) at Month 24, based on OC analysis from DB baseline.
Discussion: In this 22 month open-label extension study, treatment with lurasidone was associated with a low potential for weight gain, adverse changes in glucose and lipids, QTc effects, or clinically meaningful changes in prolactin or movement disorder symptoms. The current study represents the longest continuous exposure to lurasidone reported to date. Subjects demonstrated sustained improvement in the PANSS total score, CGI-S and MADRS for up to 24 months of treatment with flexible doses of lurasidone in the range of 40-120 mg administered once-daily.
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Disclosure: J. Cucchiaro: Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Hsu:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **M. Ogasa:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **R. Silva:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **D. Simonelli:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Pikalov:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Loebel:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **C. Correll:** Part 1: Actelion - Consultant/Advisor Alexza - Consultant/Advisor Astra Zeneca - Consultant/Advisor Biotis - Lectures Bristol Myers Squibb - Consultant/Advisor/Lectures/Research Support Cephalon - Data Safety Monitoring Board Member Eli Lilly - Consultant GSK - Lectures IntraCellular Therapies - Consultant/Advisor Johnson and Johnson/Janssen - Consultant/Research Support Otsuka - Consultant/Advisor/Lectures/Research Support Merck – Speaker's Bureau Novartis - Consultant/Advisor Pfizer - Consultant/Advisor/Lectures Sunovion - Consultant/Advisor, Part 4: Bristol Myers Squibb, Johnson and Johnson/Janssen, Otsuka.

175. Noradrenargic Involvement in Basic Information Processing Deficits in Schizophrenia: *the Effects of Clonidine on Sensorimotor Gating and Mismatch Negativity*

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Background: Cognitive deficits form core features in schizophrenia. Several studies have shown improvements in prefrontal cognitive function by α2-agonists in schizophrenia. In the present study it was investigated whether clonidine (an α2-adrenoceptor agonist) could normalize sensorimotor gating and mismatch negativity deficits in schizophrenia.
Methods: In a double blind, placebo controlled, randomized yet balanced cross-over experiment 20 male chronic patients with schizophrenia who were stable on their antipsychotic medication were assessed in an auditory mismatch negativity (MMN), prepulse inhibition (PPI), sensitization and habituation of the startle reflex paradigm on 5 occasions separated by a minimum of one week: once after oral administration of placebo and once after a single dose of either 25, 50, 75 and 150 µg of clonidine. Their results were compared with those of 20 age and gender matched healthy volunteers, who were not administered any medication.
Results: In the placebo treatment, patients showed deficient PPI and sensitization, yet normal habituation of the startle reflex compared to the controls. Dosages of 25, 50 and 75 ug of clonidine significantly increased PPI in the patients compared to placebo (respectively: $[F(1,17) = 9.7,\ p = 0.006]$, $[F(1,18) = 4.4,\ p = 0.05]$, $[F(1,18) = 9.6,\ p = 0.006]$), to such levels that it no longer differed significantly from the healthy controls. However, none of the dosages improved sensitization nor influenced habituation. Preliminary data showed that in the placebo treatment, patients had less MMN than controls, but probably due to power issues this did not reach statistical significance. Nevertheless, clonidine increased MMN to frequency deviants selectively and dose dependently up until a dose of 75 ug. However, only the increase in MMN after administration of the 75 ug dose reached statistical significance compared to the placebo treatment ($t_{38} = 2.7$, $p = 0.01$).
Discussion: The results show that PPI deficits are present even in patients with schizophrenia who are clinically stable on their antipsychotic medication. This is the first study to show that even a single low dose of clonidine added to the medical treatment of these patients not only significantly improves these PPI deficits, but even normalizes them. In addition, this is the first study to show that clonidine is able to increase MMN, dose-dependently. There appears to be a dose dependent inverted U-shaped relationship behind clonidine's effect on PPI and MMN. The data suggests that α2-agonists are potent agents to restore some of the deficiently operating fundamental basic information processes in schizophrenia patients. These results have a potentially high clinical relevance for the medical treatment of schizophrenia.
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176. Mid- And Long-Term Efficacy & Effectiveness of Antipsychotic Medications for Schizophrenia: A Data-Driven, Personalized Clinical Approach

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Background: Given that schizophrenia is a lifetime illness, mid- to long-term efficacy and safety data need to be considered to optimize outcomes. Most of the literature has been focused on acute, short-term studies. A central issue is whether in the long-run, SGAs compared to FGAs are worth the extra cost. As such, we have recently finished the first comprehensive meta-analysis of these issues.
Methods: We performed a meta-analysis of mid- to long-term double-blind outcome studies using Comprehensive Meta-analysis as well as reviewing the long-term phase of both first-episode studies and open observational trials to determine if they are consistent with our previously published quantitative meta-analysis of mostly short-term, randomized-controlled trials. In addition, we contrasted efficacy and effectiveness results with safety data to better inform the clinical management of schizophrenia.
Results: Overall, the efficacy patterns of both the controlled effectiveness and of the observational long-term, studies closely parallel the efficacy observed in the short-term, controlled studies. Surprisingly, Phase 1and 2 CATIE results are very similar to, but not identical with, the controlled short-term efficacy studies as well as to EUFEST and to naturalistic studies. These mid and long-term data suggest that olanzapine is more effective than risperidone, which are better than the other first and second generation antipsychotics – clozapine being most efficacious of all. Differences that are even larger emerged regarding the mid- and long-term safety profiles of individual antipsychotics.
Discussion: When aiming to individualize mid- to long-term schizophrenia management, antipsychotic treatment selection is complex, and the relevance and magnitude of differences among individual antipsychotics needs to be evaluated. Decisions have to be informed by available efficacy and safety from groups of patients, as well as by specific patient/family preferences and prior therapeutic and adverse effects, which drive attitudes to treatment and to compliance. Even despite intra-class differences and the complexities of antipsychotic choice, the SGAs are important contributions not only to the acute phase, but more importantly to the maintenance treatment of schizophrenia.
Disclosure: I. Glick: Part 1: Over the past three years, Dr. Glick has had research support from the NIMH, Lilly, Synovian, Pakeda, Astro-geneca, Janssen, Novartis, Otsuka, and Lundbeck. He has been on Advisory Boards for BMS, Janssen, Merck, and Novartis. He has equity in Johnson and Johnson., Part 2: He has equity in Johnson and Johnson., Part 3: Dr. Glick has had research support from the NIMH, Lilly, Synovian, Pakeda, Astro-geneca, Janssen, Novartis, Otsuka, and Lundbeck. He has been on Advisory Boards for BMS, Janssen, Merck, and Novartis., Part 4: Dr. Glick has had research support from the NIMH, Lilly, Synovian, Pakeda, Astro-geneca, Janssen, Novartis, Otsuka, and Lundbeck. He has been on Advisory Boards for BMS, Janssen, Merck, and Novartis. **J. Davis:** Part 1: Dr. Davis has nothing to disclose. **C. Correll:** None.

177. Cognitive Performance in Patients with Schizophrenia Treated with Lurasidone: Results from a Placebo- and Active-Controlled Acute Phase Study followed by a 6 Month Double-Blind Extension

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Background: The results of the large-scale CATIE study suggested that atypical antipsychotic medications may not have beneficial

effects on cognition. However, the CATIE trial specifically recruited clinically stable patients, did not include placebo controls, and did not require fixed doses of antipsychotic medications. The current active-controlled multiregional study of lurasidone in patients with schizophrenia examined cognitive functioning in initially unstable patients with schizophrenia (PANSS total score at baseline, mean = 97.4, SD = 10.5, N = 482 in ITT sample) who were then assessed at six-months in a long-term extension study.

Methods: Clinically unstable patients with schizophrenia were randomized to once-daily treatment with lurasidone 80 mg (n = 125), lurasidone 160 mg (n = 121), quetiapine XR 600 mg (n = 120) and placebo (n = 122). Subjects who completed the initial 6-week trial were eligible to enroll in a double-blind extension study involving continued treatment with flexible once-daily doses of lurasidone (40-160 mg; n = 151) or QXR (200-800 mg; n = 85). Subjects initially treated with placebo were started on flexible once-daily doses of lurasidone (40-160 mg; n = 56). Cognitive performance was examined with the computerized CogState system at acute phase baseline and after 6 weeks, 3 months and 6 months of double-blind treatment.

Results: In the acute 6-week treatment phase, task completion rates averaged 94%, but data integrity failures, based on pre-planned criteria, were noted in 23% of the cases. At 6-weeks, when the entire ITT sample was examined, there were no statistically significant differences in the CogState composite score between lurasidone dose groups, the active control and the placebo group. When patients whose data failed the prespecified integrity checks were excluded, lurasidone at 160 mg was superior on the composite cognitive functioning measure to both placebo ($p < 0.05$, d = .25) and quetiapine XR ($p < 0.05$, d = .28), while quetiapine XR, lurasidone 80 mg, and placebo did not differ from each other. UPSA-B scores were also superior to placebo at endpoint for all active treatments. The lurasidone benefit over quetiapine XR was sustained (d = .25) at the six-month endpoint.

Discussion: Secondary analyses of cases meeting prespecified criteria for validity of the data suggest a cognitive benefit for the higher dose of lurasidone compared to placebo and quetiapine treated patients over a 6-month treatment period. This is the first pharmacological study to date where the investigational treatment was superior to placebo on cognitive assessments and a functional co-primary measure, as well as demonstrating superiority to an active comparator on neurocognition. These findings will require replication, but cannot be attributed to practice effects because of the placebo corrections. Levels of data integrity failures were relatively high compared to that of previous trials that used other cognitive assessments, such as the MATRICS consensus cognitive battery.

Disclosure: P. Harvey: Part 1: Abbott Labs, Bristol-Myers-Squibb, Cypress Bioscience, En Vivo, Genentech, Johnson and Johnson, Merck and Company, Novartis, Shire, Sunovion Pharma., Part 4: Astra Zeneca Pharmaceuticals. **J. Cucchiaro:** Part 5: Sunovion Pharma. **A. Pikalov:** Part 5: Sunovion Pharma. **A. Loebel:** Part 5: Sunovion Pharma. **C. Siu:** Part 1: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 2: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche), Part 3: Paid consultant for Pfizer, Inc.; Sunovion Pharmaceuticals Inc. (Dainippon Sumitomo Pharma America); Wyeth (Now Part of Pfizer, Inc.); Memory (Now Part of Roche).

178. EVP-6124, An Alpha-7 Nicotinic Partial Agonist, Produces Positive Effects on Cognition, Clinical Function, and Negative Symptoms in Patients with Chronic Schizophrenia on Stable Antipsychotic Therapy

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Background: Patients with schizophrenia have residual cognitive deficits, even after treatment with available antipsychotic therapies. Currently, several procognitive therapies are under development, including agonists of the alpha-7 nicotinic acetylcholine (ACh) receptor (N-A7A). These receptors are located in several brain areas involved in various domains of cognition, including attention and long term and working memory. EVP-6124 is a novel, potent, and selective N-A7A agonist. Nine clinical studies with EVP-6124 have been completed in 561 unique subjects. Of these, 403 received EVP-6124 and 158 received placebo. In these studies, EVP-6124 was safe and well-tolerated and exhibited linear kinetics with a long half-life (> 60 hours) suitable for once daily dosing.

Methods: A Phase 2b study in participants with schizophrenia (n = 319) receiving stable chronic atypical antipsychotic therapy has recently been completed. The study assessed the safety and efficacy of two doses of EVP-6124 (0.3 and 1 mg once daily) versus placebo. Efficacy was evaluated by quantitative cognitive measurements using the Overall Cognition Index (OCI) from the CogState testing battery and Trails 2 and 4 of the Neuropsychological Test Battery (NTB) (all subjects), the MATRICS Consensus Cognitive Battery (MCCB) (only in a subset of subjects enrolled in the US), the Schizophrenia Cognition Rating Scale (SCoRS) and the Positive and Negative Syndrome Scale (PANSS). Statistical results, as defined in the protocol, were considered significant at $P < 0.10$ (one-sided tests).

Results: Patients with chronic stable schizophrenia, both smokers and non-smokers, treated with stable doses of an antipsychotic drug other than clozapine for at least 4 weeks before screening were treated with either placebo (n = 106), 0.3 mg/d (n = 107) or 1 mg/d (n = 106) of EVP-6124 for a total of 84 days. Participants with a score of > 4 on the Brief Psychiatric Rating Scale (PBRS) were excluded from enrollment. Approximately 54 % of the subjects were enrolled in the US. Participants were 18 to 55 years of age (both inclusive) at screening. Most of the subjects were male (68%) and white (66%).

The drug was well tolerated; there were no clinically significant findings with respect to 12 lead ECGs, vital signs, hematology, and serum chemistry evaluations or suicidal ideation and behavior. A total of 192 treatment-emergent adverse events (TEAEs) were reported in 101 (31.9%) subjects, including 25 (23.4%) subjects in the 0.3 mg dose group, 35 (33.3%) subjects in the 1 mg dose group, and 41 (39%) subjects in the placebo group. The most commonly reported TEAEs were headache (3.8%), nausea (3.2%) and nosopharyngitis (2.5%). The incidence of serious adverse events was similar among the three dosing groups; none were judged related to drug. The OCI plus Trails 2 and 4 suggested that 0.3 mg of EVP-6124, compared to placebo, was associated with improvement in general cognitive function ($P = 0.034$) and that this improvement was due mainly to the beneficial effects of the drug on visual learning, visual attention, and social cognition. The effect on the OCI (minus Trails 2 and 4) was also significantly different among the treatment groups ($P = 0.05$); further analysis indicated this was due to greater improvement in the 0.3 mg dose group compared to placebo ($P = 0.009$). This positive effect on the OCI was supported by a strong positive trend (NS) for improved

cognition on the MCCB Battery which was performed only in the US in a subset of patients (n = 166). For the 1 mg dose group, the mean change from baseline at day 84 in the overall Composite T-score and the associated percentile change, respectively (3.6;5.7), was higher than for the 0.3 mg dose group (3.0;2.6) and placebo group (1.8;2.3). Significant effects in clinical function were also seen with EVP-6124 treatment as measured by the SCoRS Interviewer Rating of clinical function. The mean change from baseline in the SCoRS Interviewer Rating, over all visits, between the 1 mg dose group and the placebo group was significant ($P = 0.065$). Improvement was also seen in the negative symptoms of schizophrenia (derived from the PANSS). For the negative subscale of the PANSS, mean decreases were greater in the 1 mg EVP-6124 group compared to the placebo group ($P = 0.028$ at end of study versus baseline; $P = 0.117$ over all visits).

Discussion: In this study, EVP-6124 treatment of subjects with stable schizophrenia resulted in improved cognition and clinical function, and decreased negative symptoms. In addition, EVP-6124 was well tolerated in this population. The beneficial effects of EVP-6124 will be further investigated in larger confirmatory studies.

Disclosure: H. Meltzer: Part 1: Dr. H Meltzer and the EVP-6124-009 Study Group received compensation from EnVivo Pharmaceuticals, Inc. for conducting some of the studies., Part 2: Dr. Meltzer received compensation from Vanderbilt University, Part 3: H Meltzer and the EVP-6124-009 Study Group received compensation from EnVivo Pharmaceuticals, Inc. for conducting some of the studies., Part 4: H Meltzer and the EVP-6124-009 Study Group received compensation from EnVivo Pharmaceuticals, Inc. for conducting some of the studies., Part 5: Vanderbilt University. **M. Gawryl:** Part 1: Employee of EnVivo Pharmaceuticals, Inc., Part 2: Employee of EnVivo Pharmaceuticals and Stock Options, Part 3: Employee of EnVivo Pharmaceuticals, Part 5: Employee of EnVivo Pharmaceuticals. **S. Ward:** Part 1: Emoployed by EnVivo Pharmaceuticals, Part 2: Employed by EnVivo Pharmaceuticals and Stock Options, Part 3: Employed by EnVivo Pharmaceuticals, Part 5: Employed by EnVivo Pharmaceuticals. **N. Dgetluck:** Part 1: Employed by EnVivo Pharmaceuticals, Part 2: Employed by EnVivo Pharmaceuticals and Stock Options, Part 3: Employed by EnVivo Pharmaceuticals, Part 5: Employed by EnVivo Pharmaceuticals. **C. Bhuvaneswaran:** Part 1: Employee fo EnVivo Pharmaceuticals, Part 2: Employee of EnVivo Pharmaceuticals and Stock Options, Part 3: Employee of EnVivo Pharmaceuticals, Part 5: EnVivo Pharmaceuticals. **G. Koenig:** Part 1: Employee of EnVivo Pharmaceuticals, Part 2: Employee of EnVivo Pharmaceuticals and Stock Options, Part 3: Employee of EnVivo Pharmaceuticals, Part 5: EnVivo Pharmaceuticals. **M. Palfreyman:** Part 1: Consultant and Contractor to Chemical Diversity, Inc and Torrey Pines Investment; Consultant to Avieneuro, NeuroNascent, Aminex. Member of Scientific Advisory Board, EnVivo Pharmaceuticals. Chairman of the Board, Amakem NV., Part 2: Consulting fees and salary from above companies and stock options at EnVivo Pharmaceuticals. Consulting fees and commisions from advsising and conducting due diligence for Torrey Pines Investment, a private equity and capital investments company making investments in biotechnology companies conducting pre-clinical and clinical studies, Part 3: Consultant and Head of Translational R&D to Chemical Diversity, Inc, a CRO conducting pre-clinical and clinical studies of new drugs., Part 4: Received grants and contracts from multiple pharmaceutical and biotechnology companies to conduct pre-clinical and clinical assessments of new drug products., Part 5: Chemical Diversity, Inc. and Torrey Pines Investment, Inc. **D. Hilt:** Part 1: Employed by EnVivo Pharmaceuticals, Part 2: EnVivo Pharmaceuticals Salary and Stock, Part 3: Employed by EnVivo Pharmaceuticals, Part 5: EnVivo Pharmaceuticals.

179. Profound Time-related Remission of Psychosis and Improvement in Cognition and Grey Matter Volume in Treatment Resistant Schizoprenia/Psychotic Spectrum Disorder During Treatment with High Dose Risperidone CONSTA: A Case Report

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Background: The concept of the psychotic spectrum disorder predated the Kraepelinean view of two distinct psychotic disorders, now known as schizophrenia (SCH) and bipolar disorder (BPD). Outcome in SCH is generally poor and usually runs a progressive downhill course with excellent outcome, after prolonged deterioriation, rare. We report here a case who illustrates the full spectrum of illness embodied in the psychotic spectrum, who after a decade of treatment resistant SCH (TRS), experienced an exceptional resolution of psychopathology and cognitive impairment, with accompanying improvement in cortical and subthalamic nucleus grey matter, unique to this case.

Methods: We have hypothesized that clozapine and some other 5-HT_{2A}-D_2antagonists are most effective in TRS at doses several multiples of doses effective in non-TRS and that these doses often require up to six months to be effective. We are currently testing this hypothesis by comparing Risperdal CONSTA 100 mg im q2 weeks vs Risperdal Consta 50 mg q 2weeks, in a double blind trial. The patient noted above consented to be in the study and was randomized to the high dose.

Results: From age 16 -18, the patient was hospitalized on two occasions for hypomanic BPD. This was followed by two decades with multiple hospitalizations for major depression with and without psychotic features and suicide attempts. Subsequently, she experienced ten years of constant severe auditory and visual hallucinations and frequent suicide attempts, during which multiple physicians in numerous clinics and hospitals diagnosed her as schizophrenic or schizoaffective. Treatment with all available typical and atypical antipsychotic drugs at standard doses, with various augmenting agents, including a 3 month course of clozapine, were without benefit. Compliance was never an issue. At initial evaluation, oral TD was present, GAF Score was 30, PANNS total 110, PANNS Positive 25, PANNS Negative 32, CGI Severity 6. Her symptoms and cognitive performance were rated the worst of 140+ patients who entered this study. 15-20% improvement was noted at week 6-18 in PANSS ratings and GAF score; CGI-S remained at 5 and her family and clinical staff noted no clinically relevant improvement. During week 22, she reported, affirmed by her husband, that upon awakening, she noted complete cessation of the decade long auditory hallucinations and visual hallucinations. She reported the return of memory, attention, and executive functioning skills, which she now claims were impaired because the severity of positive symptoms interfered with these functions. Depressive and suicidal ideas were mainly unchanged at this time. However, they subsequently improved. Cognitive testing showed performance in the $75^{Th}$to 90^{Th}percentile in most cognitive tests, e.g. Wisconsin Card Sort (executive function) and Consonant Trigrams (working memory), with scores usually at the mean or better of the rest of the sample. Depression has been minimal. This remission of symptoms, improvement in cognition, as well as a dramatic improvement in social function have persisted for the last 12 months. High resolution structural MRI images (T1-w, 1 mm isotropic resolution) were obtained from this patient and 30 other patients at baseline and 6 months, using a 3T Philips Achieva scanner. These studies revealed unique increases in gray matter in this patient near the right cingulate gyrus (Brodmann area 24) where peak z = 4.07, and bilaterally in the subthalamic nucleus with the peak z = 2.33.

The relationship between these changes and improvement in symptoms and cognition is unknown but intriguing.
Discussion: This case illustrates the plasticity of symptomatology and the evolution of psychopathology, challenges prevailing views about the independence of positive symptoms and cognition, illustrates suicidality as a separate dimension and potential for grey matter improvement, and the benefits of a prolonged trial of monotherapy with high dose of a serotonin-dopamine antagonist in TRS.
Disclosure: H. Meltzer: Part 4: Grants and consulant from Envivo, Janssen, Pfizer, Eli Lilly, ROche, Dainippon Sumitomo, Sunovion, Neurotherapeutics. **M. Sim:** None. **T. Jernigan:** None. **W. Allen:** None. **C. Cannistraci:** None. **A. Anderson:** None.

180. Antidepressant and Anxiolytic Effects of Quetiapine Strongly Correlate to Neuropeptide Y Increase and Corticotropin-releasing Hormone Decrease in CSF from Schizophrenic Patients

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Background: Cumulative data strongly indicate that neuropeptide Y (NPY) and corticotropin-releasing hormone(CRH) play a role in the CNS physiology and pathophysiology as well as in the mechanisms of action of antidepressant and antipsychotic drugs. Thus early data showed increased NPY in CSF from schizophrenic patients and modulation of NPY expression by antipsychotics in selected rodent brain regions. With regard to depression, NPY is decreased in CSF from depressed patients as well as in CNS of animal models of depression, chronic stress and PTSD. Conversely, ECT, lithium and antidepressant drugs increase NPY in CSF of depressed patients and in brain regions of rodent models. Moreover, centrally administered NPY is a potent antidepressant and anxiolytic and it rescues altered behaviors in PTSD models. In view of these findings we investigated if (1) quetiapine, originally used as antipsychotic but subsequently shown to be efficient also in major depressive disorder and both poles of bipolar disorder, would affect NPY and CRH levels in CSF of schizophrenic patients, and (2) the hypothesized effects on NPY and CRH will correlate to changes in depression and anxiety, symptoms that are common both in schizophrenia and affective disorders.
Methods: Twenty-two patients with a schizophrenic episode, mean age 35.9 years, SD ± 7.4 and mean duration of illness 20.3 months, SD ± 24.8 months participated. During the study all subjects were inpatients at the Psychiatry and Psychotherapy Department, Fulda, Germany. Structured Clinical Interview was used to confirm DSM-IV schizophrenia diagnosis. Patients were assessed with the Positive and Negative Syndrome Scale (PANSS) at baseline and at weekly intervals. Lumbar puncture (LP) was performed at baseline and again after 4 weeks of 600 mg/day quetiapine. NPY-like immunoreactivity (-LI) and CRH-LI were determined at the Karolinska Institute, Stockholm, Sweden by a person blind to the diagnosis. Repeated LPallowed calculations of differences in NPY-LI (NPY-LI) and CRH-LI (CRH-LI) levels for each individual.
Results: PANSS total score was decreased by > 20%. Quetiapine treatment was associated with a marked increase in NPY-LI and decrease in CRH-LI (ṕs <0.01). Stepwise multiple regression analysis revealed that the NPY-LI and CRH-LI levels and their ratios predicted 63% ($p < 0.001$) of the PANSS total score variability; NPY-LI42% of the PANSS anxiety items ($p < 0.05$) and CRH-LI40% of the PANSS depression items ($p < 0.05$).
Discussion: The results indicate that (1) NPY and CRH are significant determinants of clinical symptoms of depression and anxiety, (2) while the quetiapine effects on monoamines are likely related to its antipsychotic properties, the modulation of NPY and CRH accounts for its antidepressant and anxiolytic effects: Since psychiatric diagnoses are clusters of signs/symptoms of various durations and there are hardly any pathognomonic features, our approach to - independently of the underlying diagnosis - focus on specific symptoms and attempt to correlate them to biological markers and effects of drugs would seem to be a fruitful approach to elucidate the underlying neurobiology.
Disclosure: A. Mathé: None. **P. Baumann:** None. **G. Nikisch:** None.

181. Safety of Lurasidone in Short-Term Schizophrenia Trials: A Comprehensive Database Analysis

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Background: This comprehensive analysis of pooled Phase 2/3 data evaluated the safety of lurasidone in short-term schizophrenia treatment studies.
Methods: These analyses were based on combined multiregional data from seven double-blind, placebo-controlled lurasidone trials (4 with active comparators) of subjects who met DSM-IV criteria for schizophrenia with an acute exacerbation. The analysis sample consisted of subjects treated with lurasidone (daily dose range, 20-160 mg; combined total N = 1508); haloperidol 10 mg/d (N = 72); olanzapine 15 mg/d (N = 122); risperidone 4 mg/d (N = 65); quetiapine XR 600 mg/d (QXR; N = 119); and placebo (N = 708).
Results: During short-term treatment, discontinuation rates due to AEs were 7% on lurasidone, 15% on haloperidol, 7% on olanzapine, 3% on QXR, 2% on risperidone, and 5% on placebo. There were no dose-related AEs on lurasidone. At least one extrapyramidal symptom (EPS; multiple related terms were pooled) was reported by subjects treated with lurasidone (24.7%), haloperidol (54.2%), olanzapine (23.0%), QXR (7.6%), risperidone (27.7%), and placebo (9.2%). The incidence of akathisia on lurasidone was 12.9%. The proportion of subjects receiving concomitant treatment with an anticholinergic drug was 24% for lurasidone, 53% for haloperidol, 18% for olanzapine, 8% for QXR, 48% for risperidone, and 13% for placebo. The mean change in weight (kg) at LOCF-endpoint, was + 0.4 for lurasidone, + 0.0 for haloperidol, + 4.2 for olanzapine, + 2.1 for QXR, + 0.2 for risperidone, and -0.0 for placebo. The proportion experiencing ≥7% weight gain was 4.8% for lurasidone, 4.2% for haloperidol, 34.4% for olanzapine, 15.3% for QXR, 6.2% for risperidone, and 3.3% for placebo. Median LOCF-endpoint changes in lipids were: triglycerides (mg/dL), -4.0 for lurasidone, -3.0 for haloperidol, + 25.0 for olanzapine, + 4.0 for risperidone, + 9.5 for QXR, and -6.0 for placebo; total cholesterol (mg/dL), -5.0 for lurasidone, -8.0 for haloperidol, + 9.0 for olanzapine, + 6.5 for risperidone, + 6.0 for QXR, and -5.0 for placebo. Similar trends existed for changes in LDL. Median LOCF-endpoint changes in glucose (mg/dL) were similar for lurasidone (0.0) and placebo (0.0), and somewhat higher for haloperidol (+ 2.0), olanzapine (+ 4.0), risperidone (+ 3.0), and QXR (+ 3.0). Minimal-to-no changes were observed at LOCF-endpoint in HbA1c. During short-term treatment, median LOCF-endpoint changes in prolactin (ng/mL) were: -0.2 for lurasidone, + 27.6 for haloperidol, + 8.5 for olanzapine, + 0.6 for QXR, + 53.0 for risperidone, and -5.1 for placebo. In the combined lurasidone short-term database, the proportion of subjects with QTcF ≥ 60 msec was similar for lurasidone (0.3%) and placebo (0.4%); no subject treated with lurasidone had a QTcF interval > 500 msec.
Discussion: In this pooled analysis of short-term schizophrenia studies, lurasidone, in the once-daily dosing range of 20-160 mg, was associated with a relatively low incidence of extrapyramidal symptoms and a moderate incidence of akathisia. Furthermore, lurasidone was associated with a low potential for weight gain or adverse metabolic or QTc effects, and minimal effects on prolactin.

Disclosure: A. Pikalov: Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **R. Silva:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Cucchiaro:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Hsu:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **J. Xu:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc. **A. Loebel:** Parts 1-5: Full-time employee of Sunovion Pharmaceuticals, Inc.

182. D2 Receptor Occupancy Measured with 18F-Fallypride following Lurasidone Treatment in Schizophrenia Patients

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Background: Lurasidone is an atypical antipsychotic medication approved for the treatment of schizophrenia with a starting dose of 40 mg/day and maximum dose of 80 mg/day given with food. It has a Ki of 0.99 at human cloned D2 receptors and the primary activity is related to the parent compound. It reaches Cmax at 1-3 hours and plasma elimination half-life is about 18 hours. There is controversy as to whether doses higher than 80 mg per day have additional efficacy as no additional benefit of 120 mg was observed compared to 40 and 80 mg was observed in two RCT (PI and Meltzer, *et al*.,, 2011); however, 160 mg may have greater efficacy than 120 mg/day (Potkin, *et al*.,, 2010). This study examined D2 receptor occupancy and side effects after steady state lurasidone treatment. To more fully explore the time course of D2 occupancy we conducted a prospective study of schizophrenia people on steady state doses of 80, 120 and 160 mg/day lurasidone.
Methods: Twenty four patient meeting DSM-IV criteria for schizophrenia or schizoaffective disorder were washed out of their previous antipsychotic medication (5 half lives) and imaged with 18F-fallypride as a baseline measure of D2 receptors (DVR). Subjects were randomly assigned to 80, 120 or 160 mg/day and treated for about one week. Using a random assignment design subjects were scanned two or three additional times while remaining on the same dose of lurasidone subjects over the next 26 hours to create a full time activity curve for each dose group. The target times post dose were 2, 7, 14, 20 and 26 hours.
18F-fallypride PET scans were obtained with a high resolution HRRT camera. The 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 subjects 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 D2 receptors, and then contrasted with baseline washout fallypride scans. Distributed volume ratio (DVR) estimates were obtained graphically from PET scans without blood sampling using the reference region method described by Logan *et al* (1996, J Cereb Blood Flow Metab 16:834–840.) The primary endpoints, were occupancy in the caudate and putamen at various time points and lurasidone doses. Occupancy in nucleus accumbens and nonstriatal ROIs including the thalamus, and amygdala were also determined. Blood samples for lurasidone concentrations were also obtained with each PET scan. Blinded clinical assessments were obtained with PANSS, MADRS, and CGI. The ESRS measured movement side-effects effects.
Results: The average age for the first 18 subjects was 40.1 ± 9.1; 10 males and 8 females.
Discussion: These data indicate that on average high levels of D2 occupancy are maintained 24 hours after the last dose of lurasidone supporting the approved QD dosing. The full dataset is being analyzed and will be presented. In addition, the relationship between occupancy and blood concentrations of lurasidone will be described as well the relationship to movement side-effects.
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183. Effects of Metformin on Weight, Glucose, and Cognition in Chronic Psychotic Patients

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Background: Weight gain and glucose and lipid metabolic abnormalities are important side-effects of antipsychotic medications, especially many second generation antipsychotics. Metformin has been shown to be effective in preventing or reversing weight gain in first or early episode schizophrenics treated with antipsychotic drugs, but its effects on weight reduction in chronic schizophrenics treated for years with these medications is less clear or consistent. Previous research has also demonstrated that improving glycemic control, by reducing fasting plasma glucose and insulin resistance, was associated with cognitive improvement, especially in working memory, in non-psychotic subjects with type2 diabetes, but this effect has not been reported in schizophrenic patients. We conducted a retrospective study of 60 patients clinically treated with metformin for up to 12 months, and also a small open label study in a small sample of patients treated with metformin, in order to further evaluate the drug's effects on weight, metabolic parameters and cognition.
Methods: Patients had a diagnosis of schizophrenia, schizoaffective disorder, or psychotic bipolar disorder, were hospitalized in a tertiary care hospital, and were treated currently with a second generation antipsychotic medication; many

patients also had additional medications. 60 patients who had been treated with metformin for periods of 2-12 months, and had weight and metabolic data in a metabolic database were reviewed. 12 patients (with BMI35 or > than 10 lb weight gain in the past 3 months) participated in an open label study of metformin (1000-2000 mg/day for 3 months) in which fasting metabolic levels and weight related parameters were assessed monthly from baseline to 3 months; oral GTT and cognitive measured were assessed at baseline and end of study. Analysis utilized both mixed model and completer analysis for repeated measures.

Results: In the prospective study metformin produced a significant weight loss; 10 of 12 patients lost weight (mean wt change -10.1 lbs, range: +3 to -47 lbs), and metformin decreased waist circumference(mean change -2.2 in, range +2.2 to -5.5 in). There were no significant changes in metabolic levels of glucose and lipids except for a small (0.26) but statistically significant decrease in glycohemoglobin (time effect, $F = 6.31$, $P = .002$; 3 month vs. baseline $P = .006$). However, the two patients with high diabetic fasting glucose levels had a marked decrease in fasting glucose. There was no relationship between glucose changes and weight changes. There were no improvements by 3 months of metformin for scores on the RBANS, or significant changes in MATRICS Battery Working Memory scores. In the retrospective sample there were overall significant ($P < .05$) decreases in weight up to 9 months of treatment with a trend for weight decrease vs baseline at 12 months ($P = .10$). The decrease was quantitatively larger and of statistically higher significance in 25 patients with baseline BMI 35. However, overall, only 45-50% of patients showed a weight decrease 2 lbs, and 27% showed a weight increase. We are examining factors which may be associated with differential weight response to metformin.

Discussion: In this sample of chronic psychotic patients treated with atypical antipsychotics, metformin was effective in decreasing weight in many patients, and this change was not related to its glucose metabolic effects. Our data suggests that the weight reducing effects may persist for 9-12 months.

Disclosure: R. Smith: Part 4: Drug supply for acgtive and palceob varenciline form Pifzer for study supported by Stantely Meduical research Institute. **S. Dwivedi:** None. **D. John:** None.

184. The Effect of Switching from Olanzapine, Quetiapine,or Risperidone to Aripiprazole on Risk of Cardiovascular Disease: Results from the Comparison Of Antipsychotics For Metabolic Problems (CAMP) Study

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Background: Cardiovascular disease (CVD) is a major cause of premature death among individuals with schizophrenia. The Comparison of Antipsychotics for Metabolic Problems (CAMP) study, conducted by the National Institute of Mental Health-sponsored Schizophrenia Trials Network, was a multi-site, randomized controlled trial examining the strategy of switching from olanzapine, quetiapine, or risperidone to aripiprazole to ameliorate metabolic risk factors for cardiovascular disease. The primary and key secondary outcomes were non-HDL-C change and efficacy failure, respectively. The effect of switching on estimates of CVD risk were measured using changes in the Framingham Risk Score (FRS) and metabolic syndrome (MetS) status. The FRS estimates 10-year risk of "hard" coronary heart disease (CHD) (myocardial infarction and coronary death) while MetS is associated with increased risk of CVD, stroke, and diabetes mellitus.

Methods: Patients with schizophrenia or schizoaffective disorder with BMI 27 and non-HDL cholesterol (non-HDL-C) 130 mg/dl on a stable dosage of olanzapine, quetiapine, or risperidone were randomly assigned to stay on the current medication ($n = 106$) or switch to aripiprazole ($n = 109$) with 24 weeks of follow-up. After a one-month period to allow for cross-titration, laboratory tests and study assessments were conducted monthly. All study participants were enrolled in a behavioral program that promoted healthy diet and exercise. Treatments were provided openly; raters were blinded to treatment assignment.

Results: The pre-specified efficacy analyses included 89 switchers and 98 stayers who had the post-baseline measurements needed to assess changes. The least squares mean estimates of non-HDL-C decreased more for the switch than the stay groups (-20.2 vs. -10.8 mg/dl). Switching was associated with larger reductions in weight (2.9 kg) and a net reduction of serum triglycerides of 32.7 mg/dl. The analyses of FRS change found that least squares mean estimates of 10-year CHD risk decreased more for the switch (from 7.0% to 5.2%) than the stay group (from 7.4% to 6.4%). Among 129 completers, the prevalence of metabolic syndrome decreased for switchers (from 55% to 40.1%) and for stayers (from 64.2% to 58.7%).

The safety population included all participants who were randomized and received study drug ($N = 213$). Twenty-two (20.6%) switchers and 18 (17.0%) stayers experienced efficacy failure, defined in the protocol as psychiatric hospitalization, a 25% increase in the total Positive and Negative Syndrome Scale (PANSS) score, or ratings of much worse or very much worse on the Clinical Global Impression-Change Scale. Forty-seven (43.9%) switchers and 26 (24.5%) stayers discontinued the assigned antipsychotic before 24 weeks.

Discussion: In the context of a program that promoted healthy diet and exercise, both the stay and switch groups experienced reductions in indicators of CVD risk. Switching from olanzapine, quetiapine, or risperidone to aripiprazole was associated with larger reductions in risk than the behavioral program alone. The benefits of switching must be balanced against its risks, which in this study included shorter time to discontinuation of the study treatment but no significant increase in efficacy failure. In the presence of close clinical monitoring, switching from an antipsychotic with high metabolic risk to one with lower risk is an effective strategy to improve metabolic parameters and reduce risk of cardiovascular disease.

Disclosure: T. Stroup: None. **R. Hamer:** Part 1: See http://www.bios.unc.edu/~hamer/Disclosure.pdf, Part 2: I received over $10,000 from Teva in 2010 for serving as an expert witness, Part 3: See http://www.bios.unc.edu/~hamer/Disclosure.pdf, Part 4: Mpme, Part 5: No. **N. Ray:** None. **S. Esscok:** None. **J. Lieberman:** Part 1: Dr. Lieberman serves on the Advisory Board of Alkermes, Bioline, Intracellular Therapies, Eli Lilly, Pierre Fabre and PsychoGenics. He does not receive direct financial compensation or salary support for participation in research, consulting, or advisory board activities., Part 2: None, Part 3: None, Part 4: Dr. Lieberman receives grant support from Allon, F. Hoffman-La Roche LTD, GlaxoSmithKline, Eli Lilly, Merck, Novartis, Pfizer, Psychogenics, Sepracor (Sunovion) and Targacept., Part 5: N/A.

185. Schizophrenia and Co-morbid Tobacco Addiction: The Role of Impulsivity, Decision-Making and Executive Function Deficits

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Background: Cognitive performance, particular in domains such as executive function, decision-making and impulsivity, are important mediators of functional outcome (e.g., ability to hold

down a job) in persons with schizophrenia. However, patients with schizophrenia often suffer from co-morbid substance use disorders, which independent of schizophrenia are associated with increased impulsivity and poor decision-making. Interestingly, the high rates of cigarette smoking in schizophrenia (up to three times that of the general population) are thought to be an attempt to remediate the widespread cognitive deficits associated with the disorder. As such, cognitive performance in domains such as attention and processing speed is better in smokers compared to non-smokers with schizophrenia (Wing *et al*., 2011). The aim the present study was to determine whether the pro-cognitive effects of cigarette smoking extend to delay discounting (a measure of impulsivity which assesses the preference for smaller immediate rewards over future delayed rewards), decision-making and executive function deficits found in schizophrenia or alternatively if these deficits are associated with co-morbid tobacco addiction in schizophrenia. A secondary aim was to examine the relationship between these cognitive domains, as a function of smoking history (i.e., current, former and never smokers), in comparison to controls.
Methods: A cross-sectional examination of performance (N = 127) on the Kirby Delayed Discounting Task (KDDT), Iowa Gambling Task (IGT) and Wisconsin Card Sorting Task (WCST) was conducted in patients with schizophrenia (n = 66) and non-psychiatric controls (n = 61). Data were analyzed as a function of cigarette smoking history: 32 current, 12 former and 22 never smokers with schizophrenia and 23 current, 11 former and 27 never-smoking controls were assessed. Smokers were studied under satiated conditions.
Results: As expected, patients with schizophrenia performed significantly worse than controls on the IGT and WCST, but interestingly there were no differences in KDDT choices when collapsed across smoking history. However, when parsing by smoking history, differences between controls and patients with schizophrenia on the KDDT emerged: both current and former smokers with schizophrenia were significantly more impulsive than never smokers with schizophrenia, whereas, similar to other studies, control current smokers were more impulsive than former and never smokers. In contrast, decision-making and executive function deficits were not modulated by smoking history in either group. Lastly, significant correlations between KDDT, IGT and WCST measures were consistently observed in controls, whereas performance on these tasks was not correlated in patients with schizophrenia.
Discussion: The notion of beneficial effects of cigarette smoking on delay discounting, decision-making and executive function in schizophrenia were not supported in this study. Moreover, our pattern of results suggests that deficits in delay discounting appear to be trait rather than a state-dependent phenomenon in schizophrenia, which may constitute a vulnerability factor for co-morbid cigarette smoking. Our results have implications for understanding of the high vulnerability to tobacco addiction found in schizophrenia, and suggest impulsivity may play an important role in mediating this co-morbidity. Lastly, controls demonstrated robust relationships between executive function, decision-making and impulsivity, while patients with schizophrenia lacked correlations between these domains. Such an apparent disconnection between executive functioning, decision-making and impulsivity may be the result of the pathobiology associated with brain regions subserving these cognitive functions often found in patients with schizophrenia, and may pose implications for cognitive remediation and smoking cessation treatments in schizophrenia.
Disclosure: V. Wing: None. **C. Wass:** None. **R. Rabin:** None. **T. George:** Part 1: Consulting and speakers fees from Novartis, Janssen, Astra-Zeneca, Pfizer, Lilly, NIH, McGill University, Memorial University of Newfoundland, Brown University, Canadian Psychiatric Association, American Psychiatric Association, and the American College of Neuropsychopharmacology.Sit on advisory board (DSMB) for Novartis, Part 4: Pfizer, Inc.

186. Efficacy and Safety of Second- vs First-Generation Antipsychotics in First Episode Schizophrenia: A Systematic Review and Meta-analysis

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Background: Although early treatment choice in first episode schizophrenia is considered important, no meta-analysis has compared individual first-generation antipsychotics (FGAs) with second-generation antipsychotics (SGAs).
Methods: Meta-analysis of randomized, head-to-head trials comparing SGAs with FGAs in first episode schizophrenia. Primary outcomes were total psychopathology change, response rate and all-cause discontinuation. Secondary outcomes included specific-cause discontinuation, psychopathology ratings and adverse effects.
Results: Pooling data by antipsychotic class across 13 trials (n = 2509), SGAs were not different from FGAs regarding total psychopathology change, response rates, positive symptoms, Clinical Global Impressions, patient's choice discontinuation, long-term remission, and metabolic changes. Conversely, SGAs significantly outperformed FGAs regarding negative symptoms, depression, global cognition, lower discontinuation due to any cause, inefficacy and intolerability, and less EPS, akathisia, use of anticholinergics and benzodiazepines, being associated with significantly greater weight gain (p values: $<$0.05-0.01). Concerning individual SGA comparisons with an FGA, amisulpride and olanzapine outperformed FGAs in 8 and 9 out of 13 efficacy outcomes, respectively, risperidone in 4, quetiapine in 3, and clozapine and ziprasidone in 1, each. Weight gain occurred significantly more with olanzapine, clozapine and risperidone. Olanzapine caused significantly greater cholesterol increase, whereas amisulpride and ziprasidone were associated with lower triglycerides and glucose changes, respectively (p values: $<$0.05-0.01).
Discussion: Amisulpride and olanzapine and, to a lesser degree, risperidone and quetiapine were superior to FGAs in first episode schizophrenia, but weight and metabolic problems were also greater with olanzapine. Clinicians need to individualize treatment decisions, weighing different aspects of efficacy, tolerability, availability and cost.
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187. Investigation of Sex-Dependent Effects of Marijuana in Heavy Marijuana Smokers

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Background: The estimated probability of initiation of marijuana use and the subsequent risk of developing dependence is reported to vary according to sex (Wagner and Anthony, 2007). Systematic investigation of marijuana's sex-dependent effects could help to

elucidate some of the variables that contribute to these differences. There have been few reports directly assessing sex-dependent effects of marijuana. Therefore, the objective of the current study was to directly compare marijuana's subjective and cardiovascular effects in males and females matched for frequency and magnitude of current marijuana use.

Methods: Data from four studies carried out at the outpatient marijuana laboratory at the New York State Psychiatric Institute were used for this analysis. Each of these double-blind, within-subject studies measured the subjective ratings of drug quality, drug effect, mood, and physiological effects of marijuana (3.27-5.50% THC) relative to inactive marijuana (0.00%) in heavy marijuana smokers. From each study, data from equal numbers of male and female participants matched for frequency of marijuana use (days/week) and amount smoked per day (joints/day) were pooled. Subjective and cardiovascular effects of marijuana smoked according to a controlled smoking procedure were analyzed according to marijuana condition (active and inactive) and sex.

Results: Male (n = 40) and female (n = 40) participants did not differ in smoking frequency (males = 6.4 ± 1.1, females = 6.28 ± 1.2 days/week), number of joints smoked per day (males = 5.6 ± 3.5, females, = 5.04 ± 3.6), or age (males = 27 ± 5 years, females = 27 ± 6 years). However, males weighed significantly more than females (males = 74.0 ± 10.2 kg, females = 67.1 ± 10.4 kg). Preliminary findings indicate that peak positive subjective drug effect ratings including 'Good Effect,' 'Take Again,' and 'High,' were significantly elevated under active marijuana conditions relative to inactive (p 0.0001). Active marijuana also significantly increased heart rate relative to inactive marijuana (p 0.0001). Marijuana's subjective and physiological effects did not significantly differ as a function of sex; however a trend for increased ratings of 'Good Effect' in females relative to males was detected (p 0.1).

Discussion: The results from this study demonstrate that when matched for marijuana use, the behavioral effects of marijuana do not seem to differ between male and female heavy marijuana smokers. These findings suggest that sex may not contribute in a clinically meaningful manner to differences in the acute effects of marijuana in heavy smokers.

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Disclosure: Z. Cooper: None. **M. Haney:** Part 2: Teva - 2009.

188. Short-Term Modafinil Administration improves Working Memory and Sustained Attention in Cocaine-Dependent Volunteers

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Background: Long-term cocaine use is a risk factor for the onset of neurocognitive impairment in humans. One important question that remains unresolved is whether these neurocognitive deficits can be reversed or ameliorated using pharmacotherapeutics, such as modafinil. The findings from two studies of methamphetamine-dependent individuals showed that modafinil markedly enhanced performance on several measures of frontal/executive functioning (Ghahremani *et al.*, 2011; Kalechstein *et al.*, 2010). Thus, we hypothesized that modafinil would improve performance on measures of frontal/executive function in cocaine-dependent volunteers.

Methods: Eligible individuals were between 18-55 years of age, met DSM-IV criteria for cocaine dependence, primarily smoked and/or injected cocaine and were excluded for any psychiatric or medical illness. Participants were active cocaine users who were not seeking treatment at time of enrollment. Modafinil (200 mg) or placebo was administered orally once daily for 5 consecutive days. Neurocognitive measures were administered on days 0 and 5. Day 0 was selected as the baseline because, at that time, participants had been abstinent for 4-6 days (verified via urine toxicology), which minimized the likelihood that withdrawal symptoms would affect performance on cognitive performance. Day 5 was selected as the date on which to evaluate the effects of modafinil because the drug has a T-$\frac{1}{2}$ of 15 hours and, after four days of continuous administration, steady state blood levels had likely been achieved. The test administration was conducted in the following order on days 0 and 5: Hopkins Verbal Learning Test-Revised (HVLT-R; to assess verbal learning and memory), Dual N-back Task (to assess working memory), the HVLT-Delayed Recall, and finally the Continuous Performance Test-II (CPT-II; to assess sustained attention). The Wechsler Adult Intelligence Scale-III (WAIS-III; to measure intelligence) was also administered.

Results: Preliminary analyses revealed that modafinil (n = 16) and placebo (n = 14) groups did not differ with respect to basic demographic and drug use variables. Participants were primarily male and African-American,the majority were cigarette smokers, they were 43.83 ± 6.09 years old (Mean + S.D.), and self-reported using cocaine for17.73 ± 6.82 years. Primary analyses revealed that modafinil improved mean block value (n-value mean), which indicates that it improved working memory capacity so that, on average, participants were spending more time working on the 2-back versus the 1-back ($F(1,28) = 5.01$, $p = .033$). Modafinil also improved max block value (n-value max), which indicates that it improved maximum working memory capacity so that participants were spending more time working on the 2-back versus the 3-back than the 1-back ($F(1,28) = 5.11$, $p = .032$). Modafinil also improved visual block accuracy (visual accuracy), which indicates that improvement was observed vis-à-vis accuracy of responses to visual stimuli. On the CPT-II, modafinil reduced commission errors, which means that participants refrained from pressing the space bar when it was appropriate to do so ($F(1,28) = 2.51$, $p = .125$). Modafinil also reduced variability in speed of response on the CPT-II ($F(1,28) = 4.09$, $p = .053$). In addition, modafinil reduced perseverations on the CPT-II ($F(1,28) = 3.61$, $p = .068$). Finally, modafinil reduced variability in response time across different interval lengths between stimulus presentation on the CPT-II ($F(1,28) = 2.85$, $p = .102$).

Discussion: The results indicate that modafinil administration was associated with improved working memory capacity, increased vigilance, increased consistency of reaction time across a series of 400 reaction time trials, and reduced inappropriate responding in cocaine-dependent individuals. Modafinil administration did not affect the speed at which participants reacted to stimuli and did not enhance participants' capacity to remember information. Importantly, these findings show that cocaine-associated neurocognitive impairments can be remediated. It is noteworthy that these positive results were obtained in a relatively small sample and using a dosing regimen that was relatively short in duration. The potential impact of longer duration modafinil treatment on cognitive function in individuals seeking treatment for cocaine-dependence is warranted.

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189. Effects of Subjective and Objective Responses to Alcohol Challenge in Indo and Afro Trinidadians

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Background: The population of Trinidad and Tobago is composed mainly of people of East Indian (Indo-Trinidadians) and African

(Afro- Trinidadians) ancestry. Differences in alcoholism rates exist between these two ethnic groups that are explained in part by variations in the genes encoding the alcohol-metabolizing enzymes alcohol dehydrogenase (ADH) 1B and 1C. However, whether Indo Trinidadians differ from Afro-Trinidadians in level of response to alcohol has not been investigated.
Methods: In this study 50 participants, 18-25 years of age were initially screened using the Alcohol Use Disorders Identification Test (AUDIT) and the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). If they were free of any Axis I psychiatric diagnoses they were asked to participate in the alcohol challenge part of the study. Participants were given a placebo, low dose and high dose alcohol challenge and subjective and objective responses to alcohol were monitored. Blood alcohol concentrations were estimated using a breathalyzer. In addition, blood samples were collected for determination of plasma cortisol concentrations at 7 time points before and after beverage.
Results: The low dose did not cause any effects in pulse rate or performance on a rotary pursuit test whereas the high dose produced significant ($p < 0.0001$) effects on both parameters with Indo-Trinidadians displaying significantly higher alcohol induced pulse rate changes ($p < 0.03$). Participants reported significant effects of alcohol on all measures on the subjective high assessment scale (SHAS) following the high dose (p,0.0001), but at the low dose significant alcohol effects were limited to: activated ($p < 0.02$), drunk ($p < 0.004$), dizzy ($p < 0.035$), floating ($p < 0.007$), effects of alcohol ($p < 0.001$), clumsy ($p < 0.012$), and high ($p < 0.001$). Measures on the SHAS that were not significantly different between the low dose alcohol and placebo session were: uncomfortable, confused, slurred speech, nauseated, sleepy, great and terrible. Cortisol levels were unchanged following low dose alcohol challenge compared to placebo. At the high dose, alcohol appeared to produce more suppression of cortisol responses in Afro versus Indo Trinidadians. No differences in blood alcohol concentrations were found between Afro and Indo Trinidadians.
Discussion: The low dose of alcohol equivalent to 1-2 drinks caused these Afro and Indo-Trinidadian participants to feel positive effects of alcohol and to feel drunk, however, it did not produce negative subjective effects, whereas the high dose produced both positive and negative subjective effects. The low dose of alcohol also did not cause any autonomic activation as measured by heart rate or blood pressure, nor did it produce much motor impairment, whereas the high dose produced significant effects on all objective parameters. This suggests that lower doses of alcohol, that presumably do not cause "stress" as measured by cortisol and autonomic responses, may be considered low risk drinking when compared to higher drinking levels. Additionally, these pilot data suggest that Indo-Trinidadians may display more stress-like responses to high dose alcohol challenge.
Disclosure: K. Montane Jaime: None. **S. Shafe:** None. **C. Ehlers:** None.

190. Naltrexone Accentuates Ethanol-Induced Feelings of Intoxication and the fMRI BOLD Response to Negative Facial Expressions in the Insula of Treatment Seeking Alcoholics

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Background: Naltrexone (NTX) is an approved treatment for alcoholism. Evidence supports that NTX acts as a mu-opioid receptor antagonist, and blocks activation of mesolimbic dopamine neurons, ultimately resulting in lessened alcohol-induced release of dopamine in the ventral striatum. Prior work from our laboratory has shown that alcohol administration to heavy social drinkers results in an activation of the ventral striatum, as measured by fMRI BOLD. To date, there are no studies showing the effects of NTX on ethanol-induced brain activation in treatment seeking alcoholics. In this study we examined whether NTX would decrease subjective effects of an ethanol infusion and blunt the fMRI BOLD response from the ventral striatum. We also examined the effects of NTX on emotional processing, as measured by responses to angry faces on the fMRI BOLD response in various brain regions under saline/ethanol conditions.
Methods: Sixty hospitalized alcoholic patients between the ages of 21 and 50 years were randomized following detoxification, using a double blind design, to receive either 50 mg of NTX per day or placebo for 9 days. On day nine, patients underwent an fMRI scan during which time they received a 15 minute saline infusion followed by a 30 minute infusion of 6%v/v of ethanol using a physiologically-based pharmacokinetic model designed to maintain an ethanol level at approximately 0.08g%. During the saline and ethanol infusions fMRI BOLD data with and without emotional facial stimuli were collected. Rating scales were administered to quantify changes in intoxication and response to the faces.
Results: There was a significant increase in craving as a function of time ($F(1,55) = 19.79$, $p < 0.001$. There was also a significant time x treatment interaction for the rating of "Feel High" ($F(1,180) = 0.03$, $P = 0.02$). Unexpectedly, patients receiving NTX experienced higher, rather than lower, levels of subjective intoxification than subjects receiving placebo. There was no significant difference in the BOLD response in the ventral striatum between saline and ethanol infusions independent of treatment. Patients receiving the NTX showed a greater BOLD activation in response to the angry faces compared to neutral faces in the insula ($p < .005$).
Discussion: The lack of ethanol-induced BOLD response in the ventral striatum is in contrast to our previously reported results in social drinkers, but in agreement with our prior findings in heavy social drinkers. This suggests that subjects in the later stages of the addictive process may develop a degree of tolerance to the rewarding properties of ethanol. The finding that alcohol attenuated brain responses to negative emotional stimuli is consistent with its acute anxiolytic properties. Greater response to negative faces under NTX in our alcoholic subjects suggests that endogenous opioid systems are involved in emotional regulation, and that inhibiting these systems may accentuate reactivity to negative emotional stimuli.
Disclosure: D. George: None. **M. Schwandt:** None. **L. Zhang:** None. **D. Rio:** None. **R. Momenam:** None. **D. Hommer:** None. **V. Ramchandani:** None. **M. Heilig:** None.

191. Nabilone decreases Marijuana Withdrawal and Relapse in the Human Laboratory

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Background: Few individuals seeking treatment for their marijuana use achieves sustained abstinence, and no pharmacotherapy has yet been shown to improve treatment outcome. Although the synthetic cannabinoid receptor agonist, dronabinol (tetrahydrocannabinol; THC), decreases marijuana withdrawal symptoms, it has not been shown to decrease relapse in either the laboratory (Haney *et al.*, 2004, 2008) or the clinic (Levin *et al.*, 2011). Dronabinol has low bioavailability (4-20%), which may contribute to its poor clinical efficacy. The FDA-approved synthetic analog of THC, nabilone, has: 1) higher bioavailability (60-90%; Lemberger and Rubin, 1982), 2) a longer duration of action and 3) clearer

dose-linearity than dronabinol (Bedi *et al.*, submitted) . The objective of this study was to determine if maintenance on nabilone decreases marijuana withdrawal symptoms and relapse relative to placebo capsules.

Methods: Daily, nontreatment-seeking marijuana smokers (8 M, 3F), who reported smoking 8.4 ± 3.0 marijuana cigarettes/day, were enrolled in this within-subject, randomized, double-blind, study; 1 additional enrolee left the study early. Participants completed three, 8-day inpatient phases, with each phase testing a different dose of nabilone in counter-balanced order [0 mg BID, 4 mg BID, 6 mg in the AM and 0 mg in the PM]. The effect of marijuana and nabilone dose conditions on a range of behavior was measured each day. On the first inpatient day, participants repeatedly smoked experimenter-administered, active marijuana (5.6% THC). For the next 3 days, they had the opportunity to self-administer placebo marijuana (0.0% THC; Marijuana Withdrawal phase), followed by 4 days in which active marijuana was available for self-administration (Marijuana Relapse phase). Participants had to pay for self-administered marijuana using study earnings. Each inpatient phase was separated by a 7-day outpatient, washout phase.

Results: Data are preliminary, including only those who completed all phases to date ($n = 7$). *Marijuana Withdrawal Phase:* Nabilone dose-dependently ($p < 0.05$) reversed withdrawal-related sleep disruption relative to placebo, significantly increasing objective measures of sleep time, and improving ratings of 'Slept Well', and 'Fell Asleep Easily,' while decreasing ratings of 'Woke Often.' Craving for marijuana was decreased by nabilone, as were ratings of 'Irritable,' 'Angry' and 'Miserable' during withdrawal. However, nabilone increased ratings of fatigue and confusion, and worsened performance of certain cognitive tasks, particularly at the higher daily dose. Nabilone had no effect on ratings of capsule 'liking' or desire to take again relative to placebo. *Marijuana Relapse Phase:* Both active nabilone doses significantly decreased marijuana relapse ($p < 0.01$), i.e., the amount of marijuana self-administered after a period of abstinence, relative to placebo.

Discussion: These preliminary results suggest that nabilone maintenance produces robust attenuation of marijuana withdrawal symptoms, craving and relapse in daily marijuana smokers. Nabilone's long duration of action resulted in many positive significant effects even when only one active dose per day was administered. These data support clinical testing of nabilone for patients seeking treatment for their marijuana use.

Disclosure: M. Haney: Part 1: Teva Pharmaceuticals. **G. Bedi:** None. **Z. Cooper:** None. **S. Vosburg:** None. **S. Comer:** None. **R. Foltin:** Part 4: Astra-Zeneca.

192. Anhedonia predicts Diminished Sociocognitive Reward Processing in Abstinent Cigarette Smokers

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Background: Anhedonia—a trait indicative of diminished capacity to experience pleasure in response to rewards—is associated with tobacco dependence and risk of relapse during smoking cessation. One model of the mechanisms linking anhedonia and smoking postulates that high-anhedonia individuals are prone to tobacco dependence because nicotine acutely enhances reward processing by promoting mesolimbic dopamine release. Thus, high-anhedonia individuals may be better able to process and respond to rewards when smoking. If smoking is discontinued, preexisting reward processing deficits may be unmasked and exacerbated by nicotine withdrawal, which may motivate the resumption of smoking. Though this model has theoretical promise, it has received limited empirical testing. Further, it is unclear if reward processing deficits in abstinent anhedonic smokers extend to social stimuli. If so, these smokers may be less able to benefit from social support during cessation. This human biobehavioral lab study examined trait anhedonia as a predictor of sociocognitive reward processing during experimentally manipulated acute nicotine deprivation. Based on the notion that nicotine offsets reward processing deficits linked with anhedonia and that these deficits become unmasked during abstinence, we hypothesized that anhedonia would predict diminished cognitive processing of stimuli that signal social reward (i.e., happy faces) in nicotine deprived but not nondeprived states.

Methods: In a baseline session, smokers not attempting to quit ($n = 75$; 10+ cig/day) completed two anhedonia measures: the Snaith Hamilton Pleasure Scale (SHAPS) and Tripartite Pleasure Inventory—Responsiveness Scale (TPI-R). Subjects then attended two counterbalanced experimental sessions: one following 18 hours of tobacco abstinence and one after unrestricted smoking. After biological verification of abstinence or smoking, subjects completed a computerized task in which they categorized the gender of pictures of human faces expressing varying emotions (happy, angry, neutral). Greater attentional capture by a stimulus' emotional content induces more interference away from the target response (i.e., gender categorization), which slows reaction times (RTs). Thus, outcomes are interference scores (RT affectively-valenced – RT neutral trials). Mixed general linear model analysis was used with interference score as the dependent variable and between-subjects continuous anhedonia score, within-subjects deprivation status, and their interaction as predictors. Models were tested twice—once using SHAPS and once substituting TPI-R as the predictor—to examine consistency of effects across multiple anhedonia measures. Models were re-tested after adjusting for depressive symptom severity. Simple effect analyses examined the correlation between anhedonia and interference scores separately in each deprivation condition.

Results: A significant SHAPS × Deprivation interaction in predicting happiness interference scores was found before and after adjustment for depression ($ps \leq .04$, $\eta_p^2 \geq .06$). Simple effects showed a significant inverse correlation between SHAPS and happiness interference scores in the deprived condition ($r = -.28$, $p = .02$; r_p [adjusting for depression] $= -.27$, $p = .02$) and nonsignificant correlation in the nondeprived condition ($r = .08$, $p = .51$; $r_p = .09$, $p = .45$). Marginal TPI-R × Deprivation interactions for happiness interference scores were also found ($ps \leq .10$, $\eta_p^2 \geq .04$), with a significant inverse correlation for the deprived condition ($r = -.33$, $p = .004$; $r_p = -.33$, $p = .003$) but not the nondeprived condition ($r = -.10$, $p = .37$; $r_p = -.10$, $p = .38$). No significant interactions were found for anger interference scores, suggesting that the findings were specific to reward-related versus any type of affectively-valenced stimulus.

Discussion: Anhedonia predicted reduced cognitive processing of happy facial expressions, but only following acute nicotine deprivation. This finding suggests that diminished processing of reward-related cues occurs upon abstinence in high-anhedonia smokers. It is possible that this deficit may motivate reinstatement of smoking in order to remediate these deficits, and may explain anhedonia's relation to relapse risk during cessation. These results also indicate that neural pathways affected by nicotine, including the mesolimbic dopamine system, may perhaps underlie diminished reward processing in anhedonia. The mesolimbic system and sociocognitive reward processing may be fruitful targets for research and treatment of anhedonia, particularly within the context of smoking cessation.

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193. A Single Administration of Low Dose Varenicline Saturates α4β2* Nicotinic Acetylcholine Receptors in the Human Brain

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Background: Neuronal nicotinic acetylcholine receptors (nAChRs) are known to mediate tobacco reward and withdrawal in cigarette smokers. The α4β2* nAChR is the most common and widely distributed nicotinic receptor assembly in the mammalian brain. This receptor plays a critical role in the modulation of nicotine-induced dopamine release in the nucleus accumbens, known to be a hallmark of drug reward. For these reasons, researchers have aimed to pharmacologically modulate the activities of the nAChR/dopamine system to assist in smoking cessation. Varenicline (Chantix), is now considered as one of the leading medications known to significantly enhance long-term cessation in smokers attempting to quit. Acting as a partial agonist with high affinity at α4β2* nicotinic receptors, varenicline is known to modulate neurotransmitter release by binding to these receptors and, in part, blocking nicotine's actions. Over 13 million people have been prescribed varenicline worldwide, with a number of reports demonstrating a high level of success for this medication in assisting in smoking abstinence. The primary objective of this project was to determine the α4β2* nAChR occupancy in human brain of a single low dose of varenicline (0.5 mg), and to explore the relationship between receptor occupancy by varenicline and tobacco withdrawal symptoms.
Methods: Participants were six cigarette smokers who underwent two positron emission tomography (PET) sessions with the radiotracer 2-[(18)F]fluoro-A-85380 (2-FA). Prior to the PET sessions, participants were abstinent for two nights, and took an oral dose of either varenicline (0.5 mg) or matching placebo pill (double-blind) three hours prior to the bolus-plus-infusion of 2-FA (a selective α4β2* nAChR radioligand). Four hours after 2-FA infusion, subjects were scanned for 60 min, then smoked to satiety, and were subsequently scanned for another 2.5 hours in three separate blocks (40, 50, and 60 min). Measurements of plasma varenicline were made before and after the varenicline PET session. Thalamus and brainstem are the structures with the largest 2-FA binding. We determined the total distribution volume, V_T, from the placebo session prior to smoking, and the distribution volume for nonspecific binding, $V_{NS,}$ from the varenicline session after smoking. We determined the distribution volume for varenicline binding using data from the varenicline session before smoking, yielding the fractional occupancy of α4β2* nAChRs by varenicline due to a single oral administration of this medication (0.5 mg). We assessed the influence of low dose varenicline, pill placebo, and smoking to satiety on anxiety and withdrawal rating scales using the State-Trait Anxiety Inventory, Minnesota Nicotine Withdrawal Scale, Urge to Smoke-Brief, and Strength of Urge to Smoke withdrawal scales.
Results: We determined V_T to be 13.5 ± 1.2 and 9.9 ± 0.9 for thalamus and brainstem, respectively, and V_{NS} to be 5.4 ± 0.6 and 4.3 ± 0.3 for the same structures. The data are compatible with 100% occupancy of α4β2* nAChRs by varenicline, with a 90% lower limit of 90% occupancy for both thalamus and brainstem. The corresponding 90% upper limit on effective K_I with respect to plasma varenicline was 0.28 nM, compatible with the published *in vitro* result for the human cortex (0.15 nM). Smoking to satiety, but not low dose varenicline administration (versus placebo), significantly reduced tobacco withdrawal symptoms.
Discussion: Low dose varenicline occupied at least 90% (at 90% confidence) of the α4β2* nAChRs in the human brain without reducing withdrawal symptoms. We speculate that varenicline administration reduces withdrawal symptoms through a more complete and prolonged α4β2* nAChR agonism than is achieved through a single low dose.
Disclosure: S. Lotfipour: Part 1: The first author received an investigator initiated Pfizer grant for this project in 2010., Part 2: The amount of the grant received was greater than $10,000., Part 4: The first author received an investigator initiated Pfizer grant for this project in 2010., Part 5: None. **M. Mandelkern:** None. **M. Alvarez-Estrada:** Part 1: The first author (Dr. Lotfipour) received an investigator initiated Pfizer grant for this project in 2010. Part of the funding was used to hire Mr. Miguel Alvarez-Estrada as a research assistant for the study., Part 2: The grant received is greater than 10,000 per year. It provided funding for Mr. Alvarez-Estrada's salary as well as for the research., Part 3: The grant received funded Mr. Alvarez-Estrada's full time salary while working on the project, which was greater than 5% of personal income., Part 4: The first author received an investigator initiated Pfizer grant for this project in 2010. Part of the funding was used to hire Miguel Alvarez-Estrada as a research assistant for the study. **A. Brody:** Part 1: Dr. Brody is a collaborator on the current project that was funded through an investigator initiated research grant through Pfizer., Part 2: The grant received from Pfizer on this collaboration was greater than $10,000., Part 3: None., Part 4: Dr. Brody is a collaborator on the current project that was funded through an investigator initiated research grant through Pfizer.

194. The ACE Inhibitor Perindopril may Attenuate Psychostimulant Effects produced by Methamphetamine in Non-Treatment-Seeking, Methamphetamine-Dependent Volunteers

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Background: Most treatment development research for drug abuse and dependence has focused directly or indirectly on manipulating dopaminergic systems. Genetic and pharmacological evidence implicates noradrenergic mechanisms in mediating effects of stimulants, including methamphetamine. This suggests that treatments that alter noradrenergic functioning may be useful as treatments for methamphetamine dependence. The renin-angiotensin system plays an important role in regulating noradrenergic functioning. Angiotensin II (the end product of this system) potently facilitates release of norepinephrine, and this can be inhibited by treatment with angiotensin converting enzyme (ACE) inhibitors, such as perindopril. To test the hypothesis that perindopril treatment would attenuate the positive subjective effects of methamphetamine, we administered methamphetamine to non-treatment-seeking methamphetamine-dependent volunteers during treatment with perindopril or placebo.
Methods: Participants were non-treatment-seeking, methamphetamine-dependent volunteers. We used a between-subjects design in which participants were assessed during treatment with perindopril (4, 8, and 16 mg) and placebo. Perindopril tablets were over-encapsulated and placebo capsules were used to maintain the blind. Effects of methamphetamine were tested after 4-6 days of treatment with perindopril/placebo. Methamphetamine (15 and 30 mg) were administered IV on different days, paired with a dose of placebo saline to maintain the blind. Effects of methamphetamine were collected using visual-analogue scales probing for ratings of "High", "Crave Methamphetamine", "Stimulated", as well as "Good Effects", "Bad Effects", and "Like".
Results: 10-12 participants per cell, or 48 total, have completed the study to date. Participants were ~35 years of age, used methamphetamine for ~13 years, and had used the drug on ~18 of the past 30 days. Most were cigarette smokers (87%) but none met criteria for dependence on other drugs of abuse. Participants showed reductions in ratings of "Stimulated" following adminis-

tration of methamphetamine (15 and 30 mg, IV) during treatment with 8 mg perindopril, but not during treatment with the other doses. Perindopril treatment was well tolerated, and no participant experienced treatment-emergent side effects.
Discussion: These results are consistent with earlier research showing that other medications that reduce noradrenergic signaling attenuate the effects of cocaine. The non-dose-dependent effects of perindopril may be due to inhibition of the metabolism of other peptides, such as substance P. Substance P facilitates the effects of stimulants, and treatment with higher doses of perindopril may inhibit its metabolism, enhancing its effects. Alternatively, angiotensin (1-7) is an endogenous inhibitor of angiotensin II, and treatment with higher doses of perindopril may inhibit the synthesis of angiotensin (1-7), enhancing the effects of angiotensin II.
Disclosure: T. Newton: None. **R. De La Garza, II:** None. **Y. Omar:** None. **C. Haile:** None. **D. Shorter:** None. **R. Hawkins:** None. **C. Nerumalla:** None.

195. Pharmacogenetics of Naltrexone in Asian Americans: A Randomized Placebo-Controlled Laboratory Study

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Background: Recent clinical and laboratory studies have shown that the effects of naltrexone for alcoholism may be moderated by the Asn40Asp single nucleotide polymorphism (SNP) of the μ-opioid receptor gene (OPRM1). Allele frequencies for this polymorphism, however, have been shown to vary substantially as a function of ethnic background such that individuals of Asian descent are more likely to carry the minor (Asp40) allele. The objective of this study is to test the naltrexone pharmacogenetic effects of the Asn40Asp SNP in a sample of Asian Americans.
Methods: This study consists of a double-blinded, randomized, placebo-controlled laboratory trial of naltrexone. Participants (n = 35, 10 females; 13 Asn40Asn and 22 Asp40 carriers) were non-treatment seeking heavy drinkers recruited from the community. After taking naltrexone or placebo, participants completed an intravenous alcohol administration session. The primary outcome measures were subjective intoxication and alcohol craving.
Results: Results suggested that Asp40 carriers experienced greater alcohol-induced sedation, subjective intoxication and lower alcohol craving on naltrexone, as compared to placebo, and to Asn40 homozygotes. There results were maintained when controlling for *ALDH2* (rs671) and *ADH1B* (rs1229984) markers and when examining the three levels of OPRM1 genotype, thereby supporting an OPRM1 gene dose-response.
Discussion: These findings provide a much-needed extension of previous studies of naltrexone pharmacogenetics to individuals of Asian descent, an ethnic group more likely to express the minor allele putatively associated with improved biobehavioral and clinical response to this medication. These findings help further delineate the biobehavioral mechanisms of naltrexone and its pharmacogenetics.
Disclosure: L. Ray: None. **S. Bujarski:** None. **K. Miotto:** None.

196. Transdermal Alcohol Measurement for the Assessment of Naturalistic Alcohol Drinking

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Background: Common methods used to estimate current and recent alcohol consumption include self report, biochemical markers and measurement of alcohol in body fluids and breath. However, these methods have limitations in accuracy, reliability and convenience. A proportion of ingested alcohol is eliminated through the skin and can be measured at the skin surface. Transdermal alcohol concentrations are highly correlated with blood and breath alcohol concentrations. The Giner Inc. WrisTAS™ is a portable, wearable device that measures transdermal alcohol vapor using electrochemical detection. This study tested the WrisTAS device in a 7 day field trial in a naturalistic setting. Participants recorded drinking events on a daily basis in hourly time blocks. Methods were developed for interpreting the field data by two independent blinded raters and an automated computer program that analyzed the transdermal and temperature signals according to a specified set of criteria in order to identify and characterize drinking events. Self-report drinking and WrisTAS data were compared for concordance.
Methods: Thirty-three non-alcoholic, heavy drinkers wore one WrisTAS device on their wrist continuously for 7 days (except for bathing/swimming) and recorded their alcohol consumption in a daily diary. They were told to drink alcohol as usual and to not increase or decrease their use because of the device. After 7 days, the device was removed. The daily diary was reviewed and drinking confirmed. Data were uploaded into a PC and imported into an excel spreadsheet for analysis and graphing. Independent blinded raters used a specified set of criteria (13 rules) to analyze the transdermal alcohol and temperature signals and identify drinking events. The data were also analyzed by an automated rating program that incorporated the same rules. Day by day concordance between drinking events detected by the WrisTAS and self-reported drinking events recorded in the daily diary was calculated to determine true and false positive and negative drinking events. The BACCUS equation (Matthews and Miller, 1979) was used to estimate peak blood alcohol concentration (BAC) and total number of drinks in a given drinking episode.
Results: Transdermal Alcohol Sensor (TAS) peak height was correlated with estimated BAC ($R = 0.54$, $p < .0001$) calculated by the BACCUS equation from self-reported drinking. Standard drinking units (SDU),determined from the diary entries, were more strongly correlated ($R = 0.69$, $p < .0001$) with Area Under the TAS vs. Time Curve (AUC), a measure of alcohol dose. Using self report diary drinking data as the "Gold Standard," the TAS shows excellent sensitivity and specificity for determining daily drinking or not drinking. For detecting any drinking, the TAS had a sensitivity of 0.74 and a sensitivity of 0.91. For detecting 1 or more standard drinks per drinking episode, the sensitivity was 0.79 and sensitivity 0.91. The automated program yielded similar sensitivity and specificity as the human raters, 0.79 sensitivity and 0.90 specificity.
Discussion: In summary, using self report diary drinking data as the "Gold Standard," transdermal alcohol detection with the WrisTAS shows excellent sensitivity and specificity for remotely determining daily drinking or not drinking. Amount of alcohol consumed is correlated with the TAS peak height and AUC.
Disclosure: R. Swift: Part 1: Advisory Committee, D&A Pharma; Advisory Committee, Alkermes, Inc., Part 4: Eli Lilly. **A. Grenga:** None. **J. Kim:** None. **L. Tempelman:** Part 1: Director of Biotechnology Research, Giner, Inc., Part 5: Giner, Inc. **M. Moeller:** Part 5: Giner, Inc.

197. The Effect of Receptor Reserve on Allosteric Enhancement of Efficacy at Gq-coupled Muscarinic Receptors

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Background: 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. 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 spatiotemporal patterning of receptor activation. The effects of a receptor reserve on agonist potency has been studied for decades, but the effects that a receptor reserve may have on the ability of allosteric agents to enhance response has not previously been studied to our knowledge. We have developed a new method to assess the magnitude of receptor reserve associated with a particular agonist's ability to elicit a particular response. We report here on the magnitude of reserve associated with two different muscarinic responses and the influence of that reserve on the enhancement of agonist-mediated response produced by amiodarone's interaction 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), 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 were accomplished using GraphPad Prism.

Results: We have previously noted that amiodarone acts at an allosteric site on muscarinic receptors to modulate response. Here, amiodarone is shown to elevate maximal arachidonic acid (AA) release stimulated by the M_3 receptor subtype in the presence of ACh or the partial agonist pilocarpine. Amiodarone also potentiates maximal inositol phosphate (IP) metabolism stimulated by pilocarpine. Interestingly, amiodarone did not cause a similar potentiation when ACh was used to stimulate IP metabolism. The difference in amiodarone's effect on the partial and full agonist suggested that the response to ACh might be encountering a ceiling effect in the IP response. To investigate this possibility, treatment with an irreversible antagonist was used to evaluate the degree of receptor reserve associated with each response. Upon receptor inactivation, it was found that the receptor reserve for response stimulated by ACh was indeed quite large when IP metabolism was measured, but was rather modest when AA release was measured. Modifying the degree of receptor reserve also afforded the opportunity to ascertain the affinity of ACh for the receptor (K_A) in each response. ACh exhibits a higher affinity for the active state of the receptor that mediates AA release compared to the state that mediates IP metabolism. Notably, amiodarone significantly potentiates the maximal IP response stimulated by ACh after the receptor reserve is eliminated.

Discussion: Amiodarone is unusual among allosteric enhancing agents in that it increases the maximal response elicited by the orthosteric agonist without significantly affecting the potency of the agonist. The results presented here suggest that one reason for the infrequent discovery of efficacy-enhancing allosteric modulators may be that the presence of receptor reserve masks those effects. Furthermore, it is possible that such a masking effect might present a therapeutic opportunity, in that an agent with amiodarone-like properties would only enhance response in systems that lack a receptor reserve. If a pathological reduction in receptor number in certain tissues were to eliminate the receptor reserve in those tissues, the amiodarone-like agent would be enhancing in the affected tissues, but would not exert its effects in intact tissues. In conclusion, the AA and IP responses are stimulated by different conformations of the muscarinic M_3 receptor and amiodarone is capable of enhancing efficacy in both responses.

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

198. Alpha2-Adrenoceptor Blockade contributes to the Asenapine-Induced Elevation of Prefrontal Cortical Catecholamine Outflow

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Background: The novel psychopharmacological drug asenapine is approved for treatment of schizophrenia and bipolar disorder. We have previously analyzed its atypical profile using a series of well-established preclinical methods. Interestingly, asenapine was found to increase monoamine release in the medial prefrontal cortex (mPFC), which most probably contributes to its beneficial clinical profile. Mechanisms involved in the increased cortical catecholamine release were suggested to be related to e.g. antagonism at the serotonergic 5-HT2A receptor and the alpha2-adrenoceptor. Here we investigated the role of alpha2 adrenoceptor antagonism by asenapine for its effect on dopamine, noradrenaline and serotonin release in the mPFC. The alpha2-adrenergic receptors are autoreceptors, which regulate the release of both cortical noradrenaline and dopamine, as well as indirectly serotonin, are therefore of considerable interest for understanding its mode of action and can be activated by the selective alpha2 adrenoceptor agonist clonidine.

Methods: The release of monoamines was measured by using *in vivo* microdialysis in freely moving rats. The drugs were locally administered in the mPFC using reverse microdialysis. Asenapine, in a concentration without effect of its own on release of the monoamines (10 μM; administered at 0-180 min), was combined with clonidine to find out if asenapine could counteract the suppression of cortical monoamine release by the alpha2 adrenoceptor agonist.

Results: Clonidine alone (being administered between 61-120 min at 1 μM when measuring dopamine and noradrenaline and 10 μM when measuring serotonin) significantly reduced the release of dopamine, noradrenaline and serotonin (maximal effects compared to asenapine alone at the interval 61-120 are presented as area under the curve [AUC]; $63.4 \pm 11.5\%$, $53.6 \pm 10.5\%$ and $66.3 \pm 3.2\%$ [mean ± SEM], respectively; one-way analysis of variance [ANOVA] followed by planned comparison; $p < 0.001$). Furthermore, when administered simultaneously with asenapine (10 μM), the suppressant effect of clonidine on dopamine and noradrenaline release was partly but significantly reversed (AUC for dopamine and noradrenaline at the interval 150-180 min; $111.0 \pm 16.9\%$, $p < 0.01$ and $97.1 \pm 16.4\%$, $p < 0.05$, respectively).

Discussion: Although the antagonistic action of asenapine on the clonidine-induced suppression of catecholamine release in the mPFC were somewhat delayed, this effect is in all probability, at least partly, due to its alpha2 adrenoceptor blocking properties. Our results provide further insight in the mechanism of action of asenapine in the mPFC with bearing on its atypical profile. The high affinity of asenapine for 5- HT2A/2C receptors may, however, also contribute to the increased prefrontal monoamine outflow. Further studies are needed to fully clarify the mechanism of action of asenapine in the prefrontal cortex.

Disclosure: M. Marcus: None. **O. Frånberg :** None. **T. Svensson:** Part 3: AstraZeneca scientific advisory board meetings, Part 4: AstraZeneca, Organon, Schering-Plough, Merck, Johnson & Johnson.

199. The Novel ALPHA7 Receptor Partial Agonist, BMS-902483, Demonstrates Robust Efficacy across Models of Cognitive and Sensory Deficits in Schizophrenia

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Background: Cognitive impairment in schizophrenia (CIAS) continues to be inadequately treated by current antipsychotic drugs. A robust preclinical literature indicates that nicotinic alpha7 receptor agonists may provide a novel approach to treating cognitive dysfunction in schizophrenia patients. BMS-902483 was recently identified as a potent, selective, partial agonist at the alpha7 receptor. Here we report results from *in vivo* studies demonstrating that BMS-902483 i) improves object recognition memory retention in mice, ii) reverses deficits in MK-801 set shifting in the rat, iii) reverses the deficit in ID/ED performance in rats treated neonatally with phencyclidine (PCP) and iv) reverses S(+)-ketamine deficits in auditory (N40) gating in rats.
Methods: For novel object recognition (NOR) studies, male C57BL/6 mice were treated with vehicle (0.9% saline, pH 4.0; sc) or BMS-902483 (0.03-3 mg/kg, sc) 30 min prior to a 15 min training session in which the animals explored 2 identical objects. Twenty-four hours later, mice were placed back into the testing chamber containing one familiar object and one novel object and time spent exploring each object was recorded for 10 min. To confirm that efficacy was mediated through alpha7 receptor activation, BMS-902483 was also tested in mice pre-treated with NS6740 (10 mg/kg, sc; Briggs et al., 2009). Attentional set-shifting and N40 gating studies were conducted in adult Sprague Dawley rats treated with BMS-902483 (0.1-3 mg/kg, sc). Two set shift paradigms were investigated: i) reversal of acute MK-801 (0.03 mg/kg, ip) induced deficits in performance in a maze-based task (Stefani and Moghaddam, 2005) and ii) reversal of the deficits on the extra-dimensional shift (EDS) in the ID/ED task in rats treated with PCP on postnatal days 7, 9 and 11 as assessed using the digging-pot paradigm (Birrell and Brown, 2000). For N40 gating studies, the auditory-evoked response potential to ~150 paired tones was measured following vehicle or BMS-902483 treatment and following the subsequent administration of S(+)-ketamine (10 mg/kg, sc) to all animals and the gating ratio (S2/S1) calculated.
Results: In NOR studies, vehicle-treated mice had no preference for the novel object over the familiar object, while drug-treated animals spent significantly more time exploring the novel object at the MED of 0.1 mg/kg (mean ± SEM time on novel vs familiar: 26.4 ± 1.9 vs 17.0 ± 2.0 sec; $p < 0.01$). Furthermore, efficacy with BMS-902483 (0.3 mg/kg) was blocked by pretreatment with 10 mg/kg NS6740 (novel vs familiar time: 18.9 ± 2.2 vs 17.0 ± 2.1 sec; $p > 0.05$), indicating that improved object recognition is alpha7 receptor mediated. In the ID/ED paradigm, PCP-treated rats showed intact performance on the SD, CD, and IDS phases of the task, but a significant deficit in ability to perform the EDS (mean ± SEM trials to reach criteria: saline = 10.9 ± 1.2 vs PCP = 21.6 ± 2; $p < 0.001$). Administration of BMS-902483 to neonatal-PCP treated rats significantly improved EDS performance at all doses (mean ± SEM number of trials to criteria: 0.3 mg/kg = 13.0 ± 2.0**; 1.0 mg/kg = 11.9 ± 1.9**; 3 mg/kg = 9.9 ± 0.9**; **$p < 0.001$). In the maze-based set-shifting task, MK-801 impaired task performance, increasing the trials required to make an extra-dimensional rule shift compared to vehicle-treated animals (mean ± SEM number of trials to criteria: 72.0 ± 3.3 vs 49.7 ± 2.5 respectively, $p < 0.001$). BMS-902483 blocked the MK-801 deficit in set-shifting at 3 mg/kg (mean ± SEM number of trials to criteria: 54.1 ± 5.3, $p < 0.01$). Finally, S(+)-ketamine induced a deficit in N40 gating (S2/S1 ratio mean ± SEM = 1.27 ± 0.11), which was reversed by BMS-902483 at the MED of 1 mg/kg (S2/S1 ratio mean ± SEM = 0.82 ± 0.10, $p < 0.01$). Improvements in cognition and N40 gating were observed at doses producing measurable alpha7 receptor occupancy as determined by ex vivo [^{3}H]-A-585539 binding (mouse: 34–92% occupancy at 0.03 - 3 mg/kg; rat: 49–91% occupancy at 0.1–3 mg/kg).
Discussion: These results show that the alpha7 receptor partial agonist, BMS-902483, can alleviate cognitive impairment and auditory gating deficits in multiple cognition assays and preclinical models of schizophrenia. The data suggest that the compound would be an effective treatment for cognitive dysfunction in schizophrenia.
Disclosure: E. Amy: Part 5: Bristol-Myers Squibb. **R. Lidge:** Part 5: Bristol-Myers Squibb. **K. Jones:** Part 5: Bristol-Myers Squibb. **Y. Li:** Part 5: Bristol-Myers Squibb. **R. Pieschl** 5: Bristol-Myers Squibb. **S. Digavalli:** 5: Bristol-Myers Squibb. **P. Chen:** 5: Bristol-Myers Squibb. **Z. Bhagwagar:** Part 5: Bristol-Myers Squibb. **J. Cook:** Part 5: Bristol-Myers Squibb. **D. King:** 5: Bristol-Myers Squibb. **C. Iwuagwu:** 5: Bristol-Myers Squibb. **J. Macor:** 5: Bristol-Myers Squibb. **R. Zaczek:** Part 5: Bristol-Myers Squibb. **R. Olson:** Part 5: Bristol-Myers Squibb. **L. Bristow:** Part 5: Bristol-Myers Squibb.

200. *IN VITRO* Characterization of BMS-902483, a Potent, Partial Agonist at the ALPHA7 Nicotinic Acetylcholine Receptor for the Treatment of Cognitive Deficits in Schizophrenia and Alzheimer's Disease

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Background: Alpha7 nicotinic acetylcholine (nACh) receptor agonists are potential novel therapeutic agents for the treatment of cognitive and negative symptoms of schizophrenia and for the symptomatic treatment of Alzheimer's Disease (AD). The present studies describe the *in vitro* characteristics of the novel alpha7 nACh receptor partial agonist BMS-902483.
Methods: *In vitro* studies were conducted using HEK293 cells stably expressing the rat or human alpha7 nACh receptor + RIC-3, an accessory protein required for the correct folding and/or assembly of alpha7 nACh receptors at the cell surface. Functional agonist effects were demonstrated i) by Ca^{2+} fluorescence imaging in HEK/rat alpha7 cells and ii) by whole cell voltage clamp electrophysiology comparing BMS-902483-induced current responses in HEK/rat alpha7 or HEK/human alpha7 cells to those induced by acetylcholine (ACh). Alpha7 nACh receptor binding affinity was determined by the displacement of [^{125}I]-BTX or [^{3}H]-A-585539 specific binding from HEK293/human or rat alpha7 cells or rat brain membranes. BMS-902483 was also tested for i) functional agonist activity at other nicotinic receptor subtypes, namely alpha1beta1deltaepsilon (neuromuscular junction), alpha3-beta4 (ganglionic) and alpha4beta2 (CNS), ii) antagonist activity at human 5-HT_{3A} receptors, iii) pharmacological activity at 35 additional receptor/enzyme targets, and iv) inhibition of hERG currents determined by patch clamp electrophysiology. Finally, to demonstrate target binding *in vivo*, rodents were treated with vehicle (0.9% saline, pH4) or BMS-902483 (0.03–3 mg/kg, sc), the brains collected 30 min later and alpha7 nACh receptor occupancy determined by *ex vivo* [^{3}H]-A-585539 binding to brain homogenates.
Results: Radioligand binding studies showed that BMS-902483 exhibits potent binding affinity (Ki) to both rat and human alpha7 nACh receptors ([^{125}I]-BTX binding: HEK/human alpha7 = 1.25 nM, HEK/rat alpha7 = 4.4 nM; [^{3}H]-A-585538 binding: HEK/human alpha7 = 0.5 nM, rat brain = 0.52 nM). In functional assays

BMS-902483 exhibits agonist properties and induced a concentration-dependent increase in Ca^{2+} mobilization in HEK/rat alpha7 cells ($EC_{50} = 9.6 \pm 5.4$ nM). Further, whole cell voltage clamp studies showed that brief application of BMS-902483 elicits an increase in current in both HEK/rat and human alpha7 cells (EC50 = 0.14 μM and 0.24 μM respectively). BMS-902483 exhibits a partial agonist profile compared to acetylcholine; the peak current amplitude was 36% (rat) and 26% (human) and the net charge crossing the cell membrane was 60% (rat) and 62% (human) relative to the maximum effects induced by ACh. BMS-902483 exhibits no agonist activity at alpha1beta1deltaepsilon, alpha3beta4 or alpha4beta2 nACh receptors as determined by Ca2+ fluorescence imaging assays (EC50 > 100 μM). BMS-902483 shows only modest 5-HT3A receptor antagonist activity and inhibits the Ca^{2+} signal induced by 5-HT application to HEK/human 5-HT_{3A} cells (IC50 = 0.51 ± 0.18 μM). BMS-902483 inhibits hERG-induced tail currents in HEK293 cells expressing the hERG α subunit (IC50 = 3.3 μM). No additional pharmacological activities were identified at any other receptor/enzyme targets screened. Finally, *ex vivo* [^{3}H]-A-585539 binding studies show that administration of BMS-902483 to rodents produces dose- and exposure-dependent increases in alpha7 nACh receptor occupancy. The plasma and brain concentrations of BMS-902483 achieving 50% *ex vivo* alpha7 nACh receptor occupancy were as follows; rat: plasma = 5.9 nM, brain = 27.5 nM; mouse: plasma = 7.8 nM, brain = 21.9 nM.
Discussion: The present studies demonstrate that BMS-902483 is a potent, selective, partial agonist at the alpha7 nACh receptor subtype. BMS-902483 is not anticipated to produce 5-HT_3 antagonist-related gastrointestinal side effects (e.g. constipation) or QT prolongation at the low plasma exposures required for target engagement. Further BMS-902483 shows excellent brain penetration and alpha7 nACh receptor occupancy at low plasma drug concentrations in rodents. These results support the further evaluation of BMS-902483 as a novel therapeutic agent for the treatment of cognitive deficits in schizophrenia and AD patients.
Disclosure: L. Bristow: Part 5: Bristol-Myers Squibb. **N. Lodge:** Part 5: Bristol-Myers Squibb. **A. Hendricson:** Part 5: Bristol-Myers Squibb. **R. Westphal:** Part 5: Bristol-Myers Squibb. **Y. Li:** Part 5: Bristol-Myers Squibb. **R. Denton:** Part 5: Bristol-Myers Squibb. **D. Post-Munson:** Part 5: Bristol-Myers Squibb. **L. Gallagher:** Part 5: Bristol-Myers Squibb. **T. Molski:** Part 5: Bristol-Myers Squibb. **R. Pieschl:** Part 5: Bristol-Myers Squibb. **J. Cook:** Part 5: Bristol-Myers Squibb. **D. King:** Part 5: Bristol-Myers Squibb. **C. Iwuagwu:** Part 5: Bristol-Myers Squibb. **R. Olson:** Part 5: Bristol-Myers Squibb. **J. Macor:** Part 5: Bristol-Myers Squibb. **R. Zacek:** Part 5: Bristol-Myers Squibb.

201. Blockade of PramipexoleEeffects on Prepulse Inhibition and Accumbens c-Fos Expression by U99194

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Background: The preferential dopamine D3 receptor (D3R) agonist pramipexole (PPX) has been used to study the role of D3 vs. D2 receptors in the regulation of prepulse inhibition of acoustic startle (PPI) in rats, a model with clinical predictive value for antipsychotic development. We previously reported that the PPX-induced disruption of PPI is relatively insensitive to blockade by the D2-preferential antagonist L741626, suggesting a D3R-mediated mechanism of action. We also reported that PPX, but not the D2-preferential agonist, sumanirole (SUM), reduces c-Fos expression in the nucleus accumbens (NAc) with doses and time courses consistent with its effects on PPI. In the current study, we tested the sensitivity of these two effects of PPX - PPI-disruption and suppression of NAc c-Fos expression - to blockade by the D3-preferential antagonist, U99194.
Methods: Adult male Sprague-Dawley rats were administered either U99194 (0, 10 mg/kg) or L741626 (0, 1 mg/kg) and then PPX (0, 1 mg/kg) subcutaneously before placement into startle chambers for an acclimation period. Some rats completed PPI testing, while others were removed prior to acoustic stimulation, anesthetized and perfused. Brain tissue was collected and reacted with antibodies for c-Fos, and immunostaining was quantified in the NAc core (NAcC) and shell (NAcS), areas rich in D3R expression that are known to regulate PPI.
Results: ANOVA of PPI data revealed significant main effects of PPX ($F = 43.95$, df1,18; $p < 0.0001$) and U99194 ($F = 5.05$, df1,18; $p < 0.04$), and a significant interaction of (S)-PPX x U99194 ($F = 4.55$, df1,18; $p < 0.05$). Post-hoc tests confirmed that U99194 opposed the PPI-disruptive effects of PPX: among rats treated with 1.0 mg/kg (S)-PPX, PPI was significantly greater after pretreatment with 10 mg/kg vs. 0 mg/kg of U99194 ($p = 0.02$). ANOVAs of immunostaining revealed that the PPX-induced suppression of c-Fos expression in the NAc was opposed by U99194 pretreatment, but not by L741626 pretreatment. For U99194-pretreated rats, ANOVA of c-Fos expression across both NAc subregions revealed no significant effect of pretreatment ($F < 1$), but near-significant main effects of PPX treatment ($F = 4.16$, df 1,24, $p < 0.053$) and pretreatment x treatment ($F = 4.15$, df 1,24, $p < 0.053$) in the predicted directions. Post-hoc analyses showed significant c-Fos-reducing effects of PPX in animals pretreated with vehicle ($p < 0.015$) but not among those pretreated with U99194 ($F < 1$); among PPX-treated rats, c-Fos expression was arithmetically increased by U99194 pretreatment, although this effect only approached statistical significance in the NAcC ($p = 0.10$). For L741626-pretreated rats, ANOVA of c-Fos expression across both NAc subregions revealed significant main effects of pretreatment ($F = 8.98$, df 1,24, $p < 0.007$) and PPX treatment ($F = 29.14$, df 1,24, $p < 0.0001$), and a significant interaction of pretreatment x treatment ($F = 7.62$, df 1,24, $p = 0.01$). Interestingly, this interaction reflected significant c-Fos-activating effects of L741626 pretreatment among rats treated with vehicle ($p < 0.008$). Despite this activation, PPX still significantly suppressed c-Fos expression among L741626-pretreated rats ($p < 0.002$).
Discussion: Here, we demonstrate that reduction in NAc c-Fos after PPX can be selectively blocked by a D3-, but not D2-preferential antagonist. Using the same antagonist doses, PPX disruption of PPI shows similar differences in sensitivity to blockade of effects by U99194 (in the present study) but not L741626 (in our past reports). We have previously shown that PPX and the D2-selective agonist SUM both disrupt PPI in rats, but correlated decreases NAc c-Fos expression were observed with PPX but not SUM. Taken together, these findings suggest that PPX effects on PPI and NAc c-Fos expression are dependent on D3R activity. Given the known associations between antipsychotic efficacy and the ability to reverse PPI disruption and/or alter forebrain c-Fos expression, the current study suggests that manipulations of the NAc D3R system may be useful in novel antipsychotic development.
Disclosure: W. Chang: None. **M. Breier:** None. **R. Saint Marie:** None. **A. Yang:** None. **S. Hines:** None. **N. Swerdlow:** Part 1: Neurocrine, Inc. - Consultant.

202. A Deficit of Serotonin 2A Receptors Mediates the Resistance of *Egr3*-Deficient Mice to Sedation by Clozapine

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Background: The immediate early gene early growth response 3 (*EGR3*) is associated with schizophrenia and expressed at reduced

levels in the postmortem brains of patients. We have previously reported that *Egr3*-deficient (-/-) mice show heightened stress reactivity, memory and synaptic plasticity deficits, and schizophrenia-like behavioral abnormalities that can be reversed with antipsychotic treatment. We have also found that *Egr3*-/- mice display reduced sensitivity to the sedating effects of clozapine compared with wildtype (WT) littermates, a phenomenon that parallels the heightened tolerance of schizophrenia patients to antipsychotic side effects.

Methods: In the current study we have used a pharmacologic dissection approach to identify the neurotransmitter receptor that appears to mediate the resistance of *Egr3*-/- mice to sedation by clozapine. We systematically tested the locomotor response of *Egr3*-/- and littermate WT male mice to a range of drugs that target subsets of the receptors that are bound by clozapine, ultimately testing receptor subtype-specific agents. In parallel, we tested the response of these matched WT and *Egr3*-/- mice to several other first-generation and second-generation antipsychotic medications (FGAs and SGAs). To discern whether the locomotor inhibitory response to FGAs and SGAs represented sedation versus stereotypy, *Egr3*-/- and WT mice were videotaped and scored using three scales (Drowsiness, Motor Impairment, and Stereotypic Behavior) following treatment with haloperidol or clozapine. To evaluate serotonin 2A receptor ($5HT_{2A}R$) function, animals were scored for head twitch response following administration of the $5HT_{2A}R$-agonist (+/-)-2,5-dimethoxy-4-iodoamphetamine (DOI) vs. vehicle. Levels of $5HT_{2A}R$ in the prefrontal cortex of *Egr3*-/- and WT mice were determined by [^{3}H]Ketanserin binding.

Results: The FGAs haloperidol and chlorpromazine inhibited the locomotor activity of *Egr3*-/- mice at the same dosages as WT mice. In contrast, SGAs olanzapine, quetiapine, and ziprasidone mimicked the effect of clozapine by severely inhibiting the activity of WT mice at dosages that either failed to significantly affect activity of *Egr3*-/- mice, or reduced their baseline hyperactivity only to vehicle-treated WT levels. Further, in contrast to the leading theory that sedation by clozapine results from antihistaminergic effects, we show that H1 histamine receptors are not responsible for this effect in C57BL/6 mice. Instead, selective $5HT_{2A}R$ antagonists ketanserin and MDL-11939 replicate the effect of clozapine, producing sedation in WT, but not *Egr3*-/-, mice. To test whether dysfunction of $5HT_{2A}Rs$ may be responsible for this effect, we examined the head-twitch response in *Egr3*-/- mice. We found that *Egr3*-/- mice show a significantly reduced head-twitch response to the $5HT_{2A}R$-agonist DOI than their WT littermates ($p = 0.007$), consistent with a deficit in $5HT_{2A}R$ levels. [^{3}H]Ketanserin binding confirmed this finding, revealing that $5HT_{2A}R$ expression in the prefrontal cortex of *Egr3*-/- mice is reduced to one-third the level of WT controls ($p < 0.0001$), and indicating a mechanism to explain their decreased sensitivity to sedation by SGAs.

Discussion: Our findings suggest that the differential response of *Egr3*-/- vs. WT mice to sedation distinguishes SGAs from FGAs. Furthermore, this effect appears to be mediated by $5HT_{2A}Rs$, since antagonists selective for this receptor replicate the effect of clozapine on the activity of *Egr3*-/- mice, and levels of $5HT_{2A}Rs$ are significantly reduced in the forebrain of *Egr3*-/- mice. Our results are supported by a recent report that $5HT_{2A}R$-/- mice display the same resistance to locomotor activity inhibition by clozapine as *Egr3*-/- mice (*Neuropsychopharmacology* **35**(S1): S228). Together, these findings suggest that $5HT_{2A}Rs$ may also mediate at least some of the sedating properties of clozapine, and other SGAs, in humans. This is not surprising when one considers the fact that $5HT_{2A}R$-specific antagonists and inverse agonists are currently being investigated as treatments for insomnia.

Our results also have implications for the potential roles of *EGR3* and $5HT_{2A}Rs$ in influencing schizophrenia risk. Our finding that loss of *Egr3* results in significant reductions of $5HT_{2A}R$ in the brain in mice is consistent with numerous human studies showing decreased $5HT_{2A}R$ levels in the brains of schizophrenia patients. Finally, since the *HTR2A* gene, which encodes the $5HT_{2A}R$, is itself a leading schizophrenia candidate gene, these findings suggest a potential mechanism by which putative dysfunction in *EGR3* in humans may influence risk for schizophrenia.

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203. EVP-6124, an Alpha-7 Nicotinic Receptor Partial Agonist, enhances Cognition and Efflux of Dopamine, Acetylcholine, and Glutamate in Rat Cortex at Low Brain Concentrations

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Background: Stimulation of alpha-7 and alpha-4-beta-2 nicotinic acetylcholine receptors (nAChRs) is a possible method of improving some aspects of the cognitive impairments in Alzheimer's disease, schizophrenia and attention deficit hyperactivity disorder, as well as the negative symptoms in schizophrenia.

Methods: EVP-6124 is a novel partial agonist of alpha-7 neuronal nAChRs that was evaluated *in vitro* in binding and functional assays and *in vivo* in pharmacokinetic, behavioral, and microdialysis assays.

Results: In binding experiments, EVP-6124 displaced [^{3}H]-MLA (K_i = 9.9 nM, pIC_{50} = 7.65 ± 0.06) and [^{125}I]-alpha-bungarotoxin (K_i = 4.3 nM, pIC_{50} = 8.07 ± 0.04). The specificity of EVP-6124 for alpha-7 nAChRs was confirmed by the absence of displacement of [^{3}H]-cytisine from alpha-4-beta-2 nAChRs by 10 mM EVP-6124. The selectivity of EVP-6124 was further examined using a panel from MDS Pharma of more than 60 molecular targets, including peptide and non-peptide receptors, ion channels, and amine transporters. No significant interaction was found with any of the examined targets in this panel, other than at the 5-HT_3 receptor subtype. EVP-6124 inhibited the 5-HT_3 receptor by 51% at 10 nM, the lowest concentration tested. In electrophysiological studies on *Xenopus* oocytes expressing human alpha-7 nAChRs, brief pulses of EVP-6124 produced strong inward currents relative to 50 μM ACh and yielded a dose-response curve with an EC_{50} of 0.16 μM and a Hill slope of 1.6. Relative to 1280 μM ACh, EVP-6124 acted as a partial agonist (maximum amplitude of 42 ± 3%) and induced desensitization at 3 μM. In this study, the dose response curve yielded an EC_{50} of 0.39 ± 0.07 mM and a Hill coefficient of 1.45 ± 0.11. When oocytes expressing alpha-7 nAChRs were first challenged with an ACh test pulse (1280 mM) and then with a single pulse of 30 μM EVP-6124, the currents evoked by 30 mM reached up of 82 ± 7% of the current evoked by ACh. EVP-6124 had good oral bioavailability and brain penetration. EVP-6124 (0.3 mg/kg, p.o.) significantly restored memory function in scopolamine-treated rats (0.1 mg/kg, i.p.) in an object recognition task (ORT). Although subthreshold doses of donepezil (0.1 mg/kg, p.o.) or EVP-6124 (0.03 mg/kg, p.o.) did not improve memory in this task, co-administration of these subthreshold doses (p.o.) fully restored memory. In a natural forgetting model, an ORT with a 24 h retention time, EVP-6124 improved memory at 0.3 mg/kg, p.o. This improvement was blocked by the selective alpha-7 nAChR antagonist methyllycaconitine (MLA, 0.3 mg/kg, i.p. or 10 μg, i.c.v.). In micro-

dialysis experiments in freely moving rats, levels of prefrontal cortex (PFC) glutamate and dopamine were elevated by 0.1 mg/kg, s.c. of EVP-6124. Acetylcholine in PFC was elevated by 0.1 and 0.3 mg/kg. In nucleus accumbens, dopamine was slightly elevated by 0.1 and 0.3 mg/kg. These elevations were blocked by MLA at 1 mg/kg, s.c. GABA levels were unchanged by EVP-6124 in both PFC and nucleus accumbems. In order to better understand the relationship between the low, *in vivo* efficacious brain concentrations of EVP-6124 (sub- to low nanomolar range) and the *in vitro* functional EC_{50}, additional *in vitro* functional studies were performed. Sustained exposure to EVP-6124 in functional investigations in oocytes caused desensitization at concentrations greater than 3 nM, while lower concentrations (0.3–1 nM) caused an increase in the acetylcholine-evoked response.
Discussion: These actions were interpreted as representing a co-agonist activity of EVP-6124 with acetylcholine on alpha-7 nAChRs. The concentrations of EVP-6124 that resulted in physiological potentiation were consistent with the free drug concentrations in brain that improved memory performance in the ORT and induced neurotransmitter release. These data suggest that the selective partial agonist EVP-6124 improves memory performance and releases neurotransmitters by potentiating the acetylcholine response of a7 nAChRs and support new therapeutic strategies for the treatment of cognitive impairment.
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204. Discovery of The First β-Arrestin-Biased Dopamine D_2 Ligands for Probing Signaling Pathways Essential for Antipsychotic Efficacy

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Background: G protein-coupled receptors (GPCRs) signal not only via canonical pathways involving heterotrimeric large G proteins, but also via non-canonical G protein-independent interactions with other signaling proteins including, most prominently, β-arrestins. The process by which GPCR ligands differentially modulate canonical and non-canonical signal transduction pathways is a phenomenon known as "functional selectivity". Such functionally selective ligands preferentially engage either canonical or non-canonical GPCR pathways. Clearly, the discovery of ligands with discrete functional selectivity profiles will be extremely useful for elucidating the key signal transduction pathways essential for both the therapeutic actions and side-effects of drugs. However, only a small number of functionally selective GPCR ligands have been reported to date. In particular, although β-arrestin-biased ligands of G_q and G_s-coupled GPCRs are known, β-arrestin-biased GPCR ligands that selectively activate β-arrestin signaling pathways over G_i-coupled pathways have not been reported. In this presentation, we report the discovery of the first β-arrestin-biased dopamine D_2 ligands (UNC9975, UNC0006 and UNC9994) as chemical tools for probing signal transduction pathways essential for antipsychotic efficacy.
Methods: Novel analogs of aripiprazole (OPC-14597), an FDA-approved atypical antipsychotic drug, were designed and synthesized. These newly synthesized compounds were evaluated in dopamine D_2 receptor binding, D_2-mediated cAMP accumulation, and 3 orthogonal and complementary D_2-mediated β-arrestin-2 translocation assays (using the Tango, DiscoveRx, and bioluminescence resonance energy transfer (BRET) assay technologies). In addition, β-arrestin-biased compounds were evaluated in an extracellular signal-regulated kinase phosphorylation (p-ERK) reporter assay to assess their effects on β-arrestin-mediated signaling. UNC9975 and UNC9994 were tested in C57BL/6 wild-type (WT) and β-arrestin-2 knockout (βARR2 KO) mice for their ability to inhibit amphetamine- or phencyclidine-stimulated hyperlocomotion. A drug-induced catalepsy model was used to assess potential extrapyramidal side-effects of these compounds in WT and β-ARR2 KO mice.
Results: We intensely explored 4 regions of the aripiprazole template and prepared more than 150 novel compounds. Through this robust diversity-oriented modification of the aripiprazole scaffold, we discovered UNC9975, UNC0006 and UNC9994 as the first functionally selective, β-arrestin-biased dopamine D_2 ligands, which were simultaneously neutral antagonists of G_i-regulated cAMP production and partial agonists for D_2R/β-arrestin-2 interactions. In the D_2-mediated cAMP accumulation assay, UNC9975, UNC0006 and UNC9994 did not activate this G_i-coupled signaling pathway, in stark contrast to aripiprazole, which was a potent partial agonist with an E_{max} of 80%. In the 3 orthogonal and complementary D_2-mediated β-arrestin-2 translocation assays, these compounds were potent partial agonists for β-arrestin-2 recruitment to D_2 receptors. In addition, UNC9975 and UNC0006 potently activated pERK as partial agonists and co-expression of β-arrestin-2 and GRK2 significantly enhanced the efficacy of these compounds. Importantly, UNC9975 displayed potent antipsychotic-like activity without inducing motoric side-effects in inbred C57BL/6 mice *in vivo*. Genetic deletion of β-arrestin-2 simultaneously attenuated the antipsychotic actions of UNC9975 and transformed it into a typical antipsychotic drug with a high propensity to induce catalepsy. Taken together, our results suggest that β-arrestin signaling and recruitment can be simultaneously a significant contributor to antipsychotic efficacy and protective against motoric side-effects.
Discussion: We discovered the first functionally selective, β-arrestin-biased dopamine D_2 ligands through a combined medicinal chemistry and comprehensive pharmacological profiling approach. Significantly, evaluation of these novel chemical probes in WT and β-ARR2 KO mice show that β-arrestin can emerge as an important contributor to both antipsychotic drug efficacy and antipsychotic side-effects. This study represents a successful proof-of-concept for how functionally selective GPCR ligands can be discovered and validated. The β-arrestin-biased D_2 ligands discovered here are valuable tools for the biomedical community to further investigate D_2R signaling in health and disease.
Disclosure: J. Allen: None. **J. Yost:** None. **V. Setola:** None. **X. Chen:** None. **M. Sassano:** None. **M. Chen:** None. **N. Jensen:** None. **S. Peterson:** None. **P. Yadav:** None. **X. Huang:** None. **S. Frye:** None. **W. Wetsel:** None. **M. Caron:** None. **B. Roth:** None. **J. Jin:** None.

205. Opioidergic Mechanism of Body Weight Gain in Olanzapine-Treated Rats

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Background: Reduction in food intake and body weight gain (BWG) is a relatively consistent outcome of opioidergic neuro-

transmission blockade, but how these parameters are affected during pharmacotherapy with second generation antipsychotic agents, such as olanzapine (OL), has not yet been established. The aim of the present study was to examine the effects of an opioid receptor antagonist, naltrexone (NTX), on food intake and BWG in OL-treated rats.

Methods: Four groups of Wistar Han IGS rats ($n = 7$, each) were treated for 28 days with either OL, a combination of OL + NTX, NTX, or vehicle and their food intake and body weight were measured daily for the first nine days and every other day thereafter. The study outcomes were assessed by 2 x 2 factorial design with the presence or absence of OL and NTX as the two between-subject factors.

Results: The group treated with OL + NTX displayed significantly lower food intake than the OL group, but similar to the NTX- and to the vehicle-treated animals (OL x NTX interaction: $F = 12.04$, $p < 0.001$). Food intake differences were paralleled by those of body weight; (OL x NTX interaction: $F = 8.68$, $p < 0.001$), suggesting that the former may be contributory to the latter. Water intake values did not differ significantly among the groups. Plasma leptin concentrations were significantly elevated in the three groups receiving pharmacological agents (OL x NTX interaction; $F = 7.23$, $p < 0.01$), but did not differ among each other, suggesting that changes in leptin secretion and/or clearance alone would not explain the food intake and the body weight findings.

Discussion: These results are consistent with a double blind placebo controlled clinical trial recently completed by our group, and extend prior reports on anorexigenic effects of opioid antagonists by suggesting that such effects may generalize to food intake increases and body weight gain arising in the context of olanzapine pharmacotherapy.

Disclosure: D. Kurbanov: None. **P. Currie:** None. **I. Elman:** Part 4: IE has received Investigator-Initiated grants from Eli Lilly and AstraZeneca.

206. Differential Effects of AMPA Receptor Potentiators and Glycine Reuptake Inhibitors on Antipsychotic Efficacy and Prefrontal Glutamatergic Transmission.

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Background: The glutamatergic hypothesis of schizophrenia posits an impaired *N*-methyl-D-aspartate (NMDA) receptor-mediated transmission in this disease, a notion supported by postmortem findings showing a reduced prefrontal expression of several NMDA receptor subunits in schizophrenia. Accordingly, several pharmacological strategies aiming at potentiating NMDA receptor function are of considerable interest for the treatment of schizophrenia. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor positive allosteric modulators (AMPA-PAMs), Org 24448 and Org 26576, and the glycine transporter-1 (GlyT-1) inhibitor Org 25935 are developed for treatment of schizophrenia. Here we examined experimentally the ability of co-administrationof these AMPA-PAMs or the GlyT-1 inhibitor to augment the antipsychotic activity and effect on cortical NMDA receptor-mediated transmission of haloperidol, risperidone and olanzapine, respectively.

Methods: We examined antipsychotic efficacy using the conditioned avoidance response (CAR) test, extrapyramidal side effect liability using a catalepsy test, and cortical NMDA receptor-mediated glutamatergic transmission using intracellular electrophysiological recording techniques*in vitro*.

Results: Both AMPA-PAMs enhanced the suppression of CAR induced by risperidone or olanzapine and Org 24448 also enhanced the effect of haloperidol. In contrast, the GlyT-1 inhibitor did not cause any behaviorally significant effect in the CAR test. However, the GlyT-1 inhibitor, but not the AMPA-PAMs, produced a large facilitation of NMDA-induced currents. All three drugs potentiated the effect of risperidone but not haloperidol on these currents. The GlyT-1 inhibitor also facilitated the effect of olanzapine. All drugs potentiated the effect of risperidone on electrically stimulated excitatory postsynaptic potentialsin cortical pyramidal cells, whereas only the GlyT-inhibitor facilitated the effect of olanzapine.

Discussion: When comparing the two AMPA-PAMs with the GlyT-1 inhibitor tested, a slightly different clinical utility for the two types of compounds is indicated. Thus, the AMPA-PAMs may appear more useful than the GlyT-1 inhibitor as adjunct treatment to atypical APDs in order to enhance their antipsychotic efficacy, i.e. against positive symptoms in schizophrenia. On the other hand, both the AMPA-PAMs as well as the GlyT-1 inhibitor may be suitable as adjunct treatment for improving negative symptoms and cognitive impairments in this disease.

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207. Behavioral Analysis of Antipsychotic Efficacy in Beta-Arrestin2-Knockout Mice with Biased Dopamine D_2Receptor Ligands

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Background: With few exceptions, most antipsychotic drugs have efficacy at the dopamine D2 receptor (D2R) where they inhibit cAMP production. Besides this signaling pathway, it has become recognized that signaling can occur also though a G protein-independent pathway via formation of the beta-arrestin (bARR) signaling complex. Recent work has shown that most antipsychotic drugs exert effects on D2R-mediated Gi/o protein activation ranging from inverse to partial agonists and antagonists with highly variable efficacies and potencies; however, these drugs also uniformly antagonize the recruitment of bARR to the D2R. In the present study, we have examined the contribution of bARR2 in response to antipsychotic compounds.

Methods: D2R compounds were synthesized from modifications to an aripiprazole scaffold. Details regarding synthesis of the compounds, screening for binding to G protein coupled receptors, signal transduction capabilities, and D2R/bARR interactions are detailed in a companion abstract by Jin and colleagues. Behavioral effects of antipsychotic actions were evaluated in bARR2 knockout (KO) mice by treating the mice for 14 consecutive days with vehicle, clozapine, or aripiprazole and then challenging them with phencyclidine (PCP) in the open field. Using another approach, bARR-biased ligands were tested in C57BL/6 and bARR2-KO mice for their ability to inhibit amphetamine- or PCP-stimulated hyperlocomotion in the open field as well as in a catalepsy assay.

Results: In the chronic study, PCP stimulated locomotion in vehicle-treated wild type (WT) mice, whereas chronic clozapine or aripiprazole administration reduced their locomotor activities in the open field. By contrast, neither antipsychotic drug influenced the PCP-stimulated locomotion in the bARR2-KO mice. As detailed in the companion abstract by Jin and colleagues, several

bARR-biased ligands were developed that have high affinity for the D_2R. UNC9975, UNC9994, and UNC0006 displayed potent antipsychotic-like activity without motoric side-effects in inbred C57BL/6 mice *in vivo*. Genetic deletion of bARR2 simultaneously attenuated the antipsychotic actions of UNC9975 and transformed it into a typical antipsychotic drug with a high propensity to induce catalepsy.
Discussion: Together with the findings from Jin and colleagues, these results suggest that bARR2 signaling and recruitment are significant contributors to antipsychotic efficacy and they are protective against motoric side-effects. These functionally selective, bARR2-biased D_2R ligands represent valuable chemical probes for further investigations of D_2R signaling in schizophrenia, bipolar disorder, and other related conditions.
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208. The Triple Monoamine Reuptake Inhibitor, AMR-2, improves Attentional Set Shifting in a Rat Neurodevelopmental Model of Schizophrenia

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Background: The intradimensional/extradimensional (ID/ED) task assesses attentional set shifting and has cross-species application including in humans. Evidence suggests a critical role for prefrontal cortical (PFC) dopamine (DA) and norepinephrine (NE) in the acquisition and shifting of attentional sets and dysregulation of these systems may underlie the deficits in attentional set shifting seen in patients with schizophrenia and ADHD. Schizophrenia-like deficits in ID/ED performance can be modeled in neonatal rats treated with phencyclidine (PCP). The present studies have examined the ability of the triple reuptake inhibitor (TRUI), AMR-2, to reverse ID/ED performance deficits and to elevate PFC DA and NE in the rat neonatal PCP model. AMR-2 was recently identified as a potent TRUI using *in vitro* radioligand binding (Ki (nM): SERT/NET/DAT = 6.6/19.2/4.8) and functional reuptake inhibition (IC_{50} (nM): SERT/NET/DAT = 15.5/6.5/6.0) in recombinant cell lines expressing the human monoamine transporters.
Methods: Male Sprague Dawley rat pups were treated with PCP (20 mg/kg, sc) or saline on postnatal days (PD) 7, 9 and 11. All studies were conducted in adult animals between the ages of PD 56-90. ID/ED was assessed using the 2 pot digging paradigm (Birrell and Brown, 2000) which requires animals to learn the association between exemplars in one of two stimulus dimensions (odor or digging media) to correctly identify the location of a food reward. The sequence of discriminations assessed was as follows: simple discrimination (SD), compound discrimination (CD), ID shift 1 (IDS1), ID shift 2 (IDS2), ID shift 2 reversal and ED shift (EDS) with performance criteria of 6 successive correct responses required to progress at each stage. Animals were treated with vehicle (veh; 0.25% methylcellulose) or AMR-2 (0.1, 0.3 or 1 mg/kg) orally 3 hours prior to testing. Microdialysis studies were conducted in rats neonatally treated with PCP implanted with a unilateral microdialysis probe targeting the PFC (A/P + 2.5, L -0.6, V -4.0 from Bregma; Paxinos and Watson). Following overnight recovery animals were treated with vehicle or AMR-2 (0.1 or 1 mg/kg, po) and samples collected at 20 min intervals for measurement of DA or NE by HPLC with electrochemical detection.
Results: Neonatal treatment with PCP had no effect on the number of trials required to achieve performance criteria at the SD, CD, IDS 1, IDS 2 or IDS 2 reversal stages of the ID/ED task when compared to neonatal rats treated with saline. In contrast, neonatal PCP-treated rats showed a significant increase in the number of trials required to reach performance criteria at the EDS (mean ± SEM: neonatal saline treatment = 11.3 ± 1.4; neonatal PCP treatment = 22.5 ± 2*; $P<0.0001$). These results indicate that neonatal PCP-treated rats are able to acquire an attentional set but are markedly impaired when required to shift attention to a different stimulus domain. Administration of AMR-2 to neonatal-PCP treated rats significantly improved EDS performance at all doses (mean ± SEM number of trials to criteria: 0.1 mg/kg = 9.1 ± 0.9*; 0.3 mg/kg = 9.1 ± 0.9*; 1 mg/kg = 13.1 ± 1.3*; $P<0.0001$). AMR-2 also produced a sustained and dose-related elevation in extracellular levels of NE and DA in the PFC of neonatal PCP-treated rats (mean ± SEM AUC; NE: veh = 1398 ± 222, 0.1 mg/kg = 2585 ± 313*, 1 mg/kg = 3491 ± 496*; DA: veh = 1112 ± 175, 0.1 mg/kg = 2071 ± 161*, 1 mg/kg = 4901 ± 937*; *$P<0.05$).
Discussion: The present studies demonstrate that the novel TRUI, AMR-2, enhances extracellular levels of DA and NE in the PFC and alleviates ID/ED performance deficits in neonatal PCP-treated rats. These results suggest that AMR-2 may improve attentional set shifting in schizophrenia patients and may potentially improve other aspects of cognitive function that reflect impaired PFC monoamine function.
Disclosure: L. Bristow: Part 5: Bristol-Myers Squibb. **A. Easton:** Part 5: Bristol-Myers Squibb. **M. Seager:** 5: Bristol-Myers Squibb. **R. Olson:** Part 5: Bristol-Myers Squibb. **S. Liu:** Part 5: AMRI, Inc. **K. Jones:** Part 5: Bristol-Myers Squibb. **M. LaPaglia:** Part 5: Bristol-Myers Squibb. **K. Heman:** Part 5: Bristol-Myers Squibb. **Y. Li:** Part 5: Bristol-Myers Squibb. **J. Macor:** Part 5: Bristol-Myers Squibb. **B. Sargent:** Part 5: AMRI, Inc. **J. Houston:** Part 5: Bristol-Myers Squibb. **B. Molino:** Part 5: AMRI, Inc. **R. Zaczek:** Part 5: Bristol-Myers Squibb.

209. Increased Glutamate Tone in the Nucleus Accumbens mediates Excessive Ethanol Drinking in Dependent Mice

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Background: Chronic ethanol exposure is known to produce alterations in brain glutamate activity. Using a mouse model of ethanol dependence and relapse drinking, we have shown that repeated cycles of chronic intermittent ethanol (CIE) exposure produces robust escalation of voluntary ethanol drinking. This study examined the effects of CIE exposure on extracellular glutamate levels in the nucleus accumbens (NAc) and how manipulation of glutamatergic tone in this brain region influences ethanol consumption in the context of dependence.
Methods: After implanting bilateral guide cannulae positioned above the NAc, mice were trained to drink ethanol in a 2-bottle choice (15% v/v vs. water), limited access (2 hr/day) paradigm. After establishing stable baseline ethanol intake, mice received 4 weekly cycles of chronic intermittent exposure (16 hr/d for 4d) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers, with each exposure cycle alternating with a week of limited access drinking test sessions.

Using *in vivo* microdialysis procedures, extracellular glutamate levels were measured in dialysate samples collected from EtOH and CTL mice at the end of the fourth drinking test period or after an extended week of drinking (6-7 vs. 12-14 days after final CIE exposure) by HPLC with fluorescence detection. In separate animals, the nonselective glutamate transporter blocker TBOA was microinjected in the NAc to examine effects on extracellular glutamate levels (via dialysis probe) and drinking in EtOH and CTL groups of mice. Also, Western blot analyses were performed using a cross-linking procedure to examine surface vs. intracellular expression of the primary sodium-dependent glutamate transporter GLT-1 (EAAT2) in EtOH and CTL groups.

Results: As expected, voluntary ethanol consumption significantly escalated in EtOH mice above their baseline level of intake and in comparison to non-dependent CTL mice over successive CIE cycles (3.64 ± 0.30 vs. 2.70 ± 0.16 g/kg during final Test; $p < 0.05$). Microdialysis data revealed significantly higher baseline extracellular glutamate levels in NAc of EtOH mice ($N = 13$) compared to CTL mice ($N = 14$) at 6-7 days following final CIE exposure (1.43 ± 0.25 μM vs. 0.69 ± 0.13 μM; $p < 0.05$), a ~107% increase. In separate groups of EtOH ($N = 8$) and CTL ($N = 7$) mice a similar effect was observed at 12-14 days following final CIE exposure, i.e., extracellular glutamate levels were ~114% higher in EtOH mice compared to CTL mice ($p < 0.05$). Microinjection of TBOA (0, 250, 500 μM; 0.25 μL/min) into the NAc during the fourth drinking test period (30 min prior to start of 2 hr drinking session) increased ethanol drinking in a dose-related manner in both EtOH and CTL groups ($p < 0.05$), and this effect was greater in EtOH compared to CTL mice ($p < 0.05$). That is, EtOH mice injected with 250 and 500 μM TBOA evidenced a 52% and 68% increase in ethanol intake above drinking produced by vehicle infusions, while these TBOA doses produced 22% and 41% increases in drinking in CTL mice. Ethanol consumption returned to respective pre-TBOA (or vehicle) levels for EtOH and CTL groups the day following microinjections. Reverse perfusion of 125-500 μM TBOA increased extracellular glutamate levels in the NAc in a dose-related fashion ($p < 0.05$), with 500 μM TBOA resulting in an initial 46 ± 18% increase before gradually decreasing over 45-60 min to baseline levels. Finally, preliminary evidence indicates no difference EtOH and CTL groups in EAAT2 levels (surface and total expression) in NAc.

Discussion: These results indicate that excessive levels of QJ;drinking associated with repeated cycles of CIE exposure is accompanied by significantly elevated extracellular glutamate levels in the NAc, and this effect persisted well beyond acute withdrawal (at least 12-14 days following final CIE exposure). Microinjection of TBOA into the NAc increased extracellular glutamate levels as well as elevated ethanol consumption in a dose-related manner, with the effect on drinking more robust in EtOH compared to CTL mice. Collectively, these data suggest that ethanol dependence produces enduring changes in glutamate activity in the NAc and that increased glutamate tone in the NAc may promote/drive, at least in part, excessive ethanol drinking associated with dependence. While alterations in EAAT2 expression do not appear to underlie this effect, other mechanisms that play a role in regulating glutamate homeostasis in the NAc are currently being examined in this CIE model of ethanol dependence and relapse drinking.

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Disclosure: H. Becker: Part 1: Eli Lilly and Company, Part 4: Eli Lilly and Company. **W. Griffin:** None. **V. Ramachandra:** None. **P. Mulholland:** None.

210. Buspirone reduces the Reinforcing Effects of Cocaine and Cocaine + Nicotine Polydrug Combinations in Rhesus Monkeys

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Background: Cocaine abuse and nicotine dependence are major public health problems that continue to challenge medication-based treatment. Most cocaine abusers also smoke cigarettes, so an ideal pharmacotherapy would target concurrent cocaine and nicotine abuse. Buspirone (Buspar®) is a clinically available, non-benzodiazepine anxiolytic medication thought to act on serotonin and dopamine systems. Buspirone has been evaluated as an anti-smoking medication, and in preclinical studies, acute buspirone administration reduced cocaine self-administration by rhesus monkeys.

Methods: The present study evaluated the effectiveness of chronic buspirone treatment on self-administration of cocaine and cocaine + nicotine combinations. Four adult male rhesus monkeys *(Macaca mulatta)*were trained to self-administer cocaine (0.1 mg/kg/inj) and food (1 g banana-flavored food pellets) during four 1 hr daily sessions under a second-order schedule of reinforcement (FR2 [VR16:S]). Buspirone (0.10 to 0.56 mg/kg/hr) was administered intravenously through one lumen of a double lumen catheter every 20 min for 23 hours each day, for at least 7 consecutive days. Each buspirone treatment was followed by saline-control treatment for at least 5 days or until food- and drug-maintained responding returned to baseline levels.

Results: Buspirone dose-dependently reduced responding maintained by cocaine and cocaine + nicotine combinations. Buspirone's effects on food-maintained responding were variable. Buspirone was most selective on the ascending limb of the cocaine dose-effect curve and during cocaine + nicotine self-administration.

Discussion: These preliminary findings indicate that buspirone selectively attenuates the reinforcing effects of cocaine and cocaine + nicotine polydrug combinations in a nonhuman primate model of drug self-administration. Studies of the effects of buspirone on nicotine self-administration are in progress.

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Disclosure: N. Mello: None. **J. Bergman:** None. **P. Fivel:** None. **S. Kohut:** None.

211. Methamphetamine Self-Administration attenuates the Persistent Dopaminergic Deficits caused by a Subsequent Methamphetamine Treatment

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Background: Clinical studies have demonstrated that abstinent methamphetamine (METH) abusers display persistent dopaminergic deficits. More recently, preclinical studies involving rats have demonstrated that METH self-administration likewise affects dopaminergic neurons. In particular, METH self-administration decreases striatal dopamine transporter uptake and/or immunoreactivity as assessed 8 or 30 d after the last self-administration session. The impact of self-administration on a subsequent high-dose "binge" METH treatment is unknown.

Methods: Rats underwent 7 d of self-administration (8 h/session; 0.12 mg/infusion) during the light cycle. For each active lever press, an infusion pump (Coulbourn Instruments) connected to a liquid swivel (Coulbourn Instruments) suspended outside the operant chamber delivered 10 ul of METH or saline per infusion over a 5-s duration through a polyethylene tube located within a spring leash

(Coulbourn Instruments) tethered to the rat. During this period, both levers were retracted. Following the infusion, the levers remained retracted for an additional 20 s. The active lever was counterbalanced within each group. Pressing the inactive lever resulted in no programmed consequences although it was recorded. Rectal temperatures were measured using a digital thermometer (Physiotemp Instruments, Clifton, NJ) approximately 30 min after the end of each self-administration session. Twenty-four hours after the final session, rats received 4 injections (s.c.) of METH (7.5 mg/kg/injection, s.c. 2-h intervals; referred to herein as a "binge" treatment) or saline (1 ml/kg/injection) and were sacrificed 7 d later. Rectal temperatures were recorded throughout the binge METH exposure.
Results: METH self-administration attenuated both the hyperthermia and the dopaminergic deficits caused by a subsequent, repeated high-dose binge METH exposure.
Discussion: This finding is of potential clinical relevance, as the resistance to persistent deficits caused by the METH self-administration paradigm may explain findings that the magnitude of deficits in human METH abusers is often less than deficits reported in preclinical models. Thus, these results may help elucidate mechanisms underlying the neurochemical deficits observed in human METH abusers.
Disclosure: L. McFadden: None. **G. Hadlock:** None. **P. Vieira-Brock:** None. **G. Hanson:** None. **A. Fleckenstein:** None.

212. Src Tyrosine Kinase-Mediated Activation of NMDA Receptor Function in the Dorsal Hippocampus is Necessary for Drug Context-induced Cocaine-seeking Behavior in Rats

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Background: Exposure to a cocaine-associated environmental context can elicit craving and relapse in cocaine users and drug-seeking behavior in laboratory animals. Previous studies from our laboratory have shown that the dorsal hippocampus plays a critical role in drug context-induced cocaine-seeking behavior; however, the neuropharmacological and intracellular mechanism of this phenomenon are not well understood. The present study tested the hypothesis that NMDA glutamate receptor stimulation in the dorsal hippocampus is necessary for drug context-induced cocaine-seeking behavior and this event may require the activation of NMDA receptors by the Src family of tyrosine kinases (Src). Additional experiments examined the hypothesis that D1 dopamine receptor stimulation and mGlu1 metabotropic glutamate receptor stimulation, known activators of Src, are also needed for this behavior.
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 the test day, the NMDA antagonist, AP-5 (0, 1.25, or 2.5 μg), the Src inhibitor, PP2 (0, 6.25, or 62.5 ng), the dopamine D1 receptor antagonist, SCH 23390 (0, 0.1, or 1.0 μg), or the mGlu1 receptor antagonist, JNJ16259685 (0, 0.6, 30, 120 pg) was microinfused bilaterally into the dorsal hippocampus. The effects of these manipulations were assessed on reinstatement of extinguished cocaine-seeking behavior (i.e., non-reinforced lever presses) in the previously cocaine-paired and extinction contexts. The effects of PP2 on NR2b subunit phosphorylation in the dorsal hippocampus were assessed using quantitative Western immunoblotting. Furthermore, the effects of all manipulations were evaluated on general motor activity in a novel context and on food-reinforced instrumental behavior to assess possible rate-limiting effects.
Results: Re-exposure to the previously cocaine-paired context, but not the extinction context, reinstated extinguished cocaine-seeking behavior in vehicle-pretreated rats. Intra-dorsal hippocampal pretreatment with AP-5, PP2, SCH23390, or JNJ16259685 dose-dependently attenuated drug context-induced cocaine-seeking behavior without altering lever responding in the extinction context, general locomotor activity, or food-reinforced instrumental behavior. Furthermore, PP2 pretreatment reduced phosphorylated NR2b levels in the dorsal hippocampus.
Discussion: The present findings suggest that NMDA, D1 dopamine, and mGlu1 receptor stimulation as well as Src activation are necessary for drug context-induced cocaine-seeking behavior. One possible explanation for this pattern of findings is that dopamine D1 or mGlu receptor stimulation may lead to the activation of Src, the subsequent phosphorylation of NR2b subunits, and the facilitation of NMDA-receptor mediated processes that effect drug context-induced incentive motivation for cocaine.
Disclosure: X. Xie: None. **A. Arguello:** None. **A. Wells:** None. **H. Lasseter:** None. **R. Fuchs:** None.

213. Effects of Neurotensin (NT) Systems on *Maintenance*, *Extinction* and *Reinstatement* of Methamphetamine (METH) Self-Administration (SA)

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Background: The personal and social damage caused by METH abuse/addiction is due, at least in part, to its profound actions on basal ganglia and limbic dopamine (DA) systems. However, there are currently no approved medications for METH abuse or addiction, including drugs that directly influence dopaminergic functions. Consequently, many investigators study the role of non-dopaminergic systems in METH abuse to identify potential therapeutic targets. Of particular interest to our group is NT, a tridecaneuropeptide that is prominent in basal ganglia and limbic structures. In the basal ganglia, NT is associated with striatal efferent feedback pathways that project to both the substantia nigra and globus pallidus and contribute to the regulation of DA-related functions. Similar, but less-well defined, NT feedback pathways appear to also project from the nucleus accumbens and help regulate limbic dopaminergic functions.
Methods: METH dependence was modeled in male Sprague Dawley rats using a self-administration (SA) protocol. Animals were initially trained to lever press in operant chambers for food pellets after which jugular cannulae were surgically implanted. Following recovery, the lever-pressing activity was associated with i.v. infusions of METH (0.06 mg/infusion) for 4-h daily sessions. Stable lever-pressing developed within 3 d and is referred to as *maintenance*. During *extinction* sessions, rats were placed in their operant chambers, but lever pressing resulted in saline, and not METH, infusions: after an initial surge of lever-pressing, these conditions resulted in elimination of lever pressing after 5 sessions. After *extinction*, some rats were exposed to a single infusion of METH and the lever pressing was again associated with METH infusions as described above for *maintenance*. This resulted in resumption of lever pressing and is referred to as *reinstatement*.
Results: Substitution of the NT agonist PD149163 for METH in the infusion solution during *maintenance*, rapidly and dramatically stopped lever pressing without altering normal motor behavior. We also observed that subcutaneous injections of PD149163 stopped the enhanced lever-pressing behavior that occurred in rats during their first day of *extinction* and PD149163 blocked lever pressing during the first day of drug-induced *reinstatement* of

METH SA. Finally, we examined the effects of the selective NT antagonist SR48692 and found that this compound prevented elimination of lever pressing during *extinction* but had no effect on SA of METH during either *maintenance* or *reinstatement*.

Discussion: Overall NT is thought to have an inhibitory influence on the stimulatory and reward properties of psychostimulants. This was confirmed by our findings that substitution of the NT agonist PD149163 for METH during *maintenance*, rapidly and dramatically stopped lever-pressing behavior while not altering normal motor behavior. In addition, PD14163 reduced lever pressing associated with early *extinction* and *reinstatement*. Our results suggest that (i) systemic activation of NT receptors diminishes contingent behavior in general, and (ii) systemic NT agonists are themselves not self-administered and they do not substitute for psychostimulants such as METH. In addition, we discovered that endogenous NT systems play an important role in *extinction* of METH SA. *This work was supported by U.S. Public Health Service Grants, DA00378, DA11389 and DA019447, DA004222, DA13367.*

Disclosure: A. Hoonakker: None. **M. Alburges:** None. **G. Hanson:** None.

214. Ceftriaxone increases Glutamate Transport and Basal Glutamate Levels in the Nucleus Accumbens Core of Cocaine Self-Administering Animals

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Background: Withdrawal from cocaine self-administration is associated with altered glutamate homeostasis in the nucleus accumbens core. Relative to control animals, basal levels of extracellular glutamate are reduced in withdrawal while reinstatement is accompanied by increased glutamate levels. We have previously shown that following cocaine self-administration and 2-3 weeks of withdrawal, levels of sodium-dependent glutamate uptake and expression of the major glial glutamate transporter GLT-1 are significantly decreased relative to yoked-saline controls. Ceftriaxone, a member of the beta-lactam family of antibiotics, has been shown to increase both GLT-1 and glutamate uptake. We have shown that chronic ceftriaxone treatment during extinction training significantly attenuates both cue- and cocaine-induced reinstatement and prevents the decrease in GLT-1 protein levels observed following cocaine self-administration. Here the effects of ceftriaxone on basal glutamate levels and glutamate uptake in cocaine self-administering animals and yoked-saline controls were investigated.

Methods: Male Sprague-Dawley rats underwent two weeks of cocaine self-administration (or were assigned as yoked-saline controls) followed by two weeks of extinction training. During the last week of extinction training, half of all cocaine and saline animals received ceftriaxone (200 mg/kg IP) while the remaining half received saline injections. Following seven daily treatments with ceftriaxone or vehicle, a subset of animals were probed for no-net-flux microdialysis. On the next day, increasing concentrations of glutamate (0, 2.5, 5, 10 uM) were infused through the dialysis probe and the efflux was collected. At the same experimental timepoint, a second set of animals was sacrificed, the nucleus accumbens core dissected and used in a tritiated-glutamate uptake assay. A third group of animals underwent cocaine self-administration and were treated with either Ceftriaxone or Vehicle for seven days and also received intra-accumbens infusions of Vivo-Morpholinos to knock-down GLT-1 expression (or a control peptide) and were tested for cue-primed reinstatement.

Results: Both cocaine and yoked-saline animals treated with ceftriaxone displayed higher basal glutamate levels than vehicle-treated animals. Ceftriaxone prevented cocaine from decreasing both sodium-dependent and -independent glutamate transport. Ceftriaxone-treated animals which received GLT-1 knockdown significantly reinstated cocaine-seeking in response to cue presentation while those receiving intra-accumbens infusion of the inactive peptide displayed attenuated reinstatement relative to vehicle-treated controls.

Discussion: These results indicate that ceftriaxone increases both glutamate uptake and export via system x_C-. Because the knock-down of GLT-1 prevented ceftriaxone from attenuating reinstatement, it is possible that ceftriaxone's ability to increase glutamate uptake is the primary mechanism by which it attenuates the reinstatement of cocaine-seeking.

Disclosure: L. Knackstedt: None. **K. Reissner:** None.

215. Progressive Behavioral Supersensitivity to Nicotine during Early Withdrawal from Chronic Cocaine Administration and Prevention of Cocaine Sensitization using Mecamylamine

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Background: Cocaine abusers smoke tobacco products more often than non-cocaine users. Interestingly, nicotinic receptors are postulated to modulate development and consolidation of behavioral sensitization during early withdrawal from intermittent cocaine administration.

Methods: Male Sprague-Dawley rats were pretreated with single saline (2 ml/kg, s.c.) or cocaine injections (40 mg/kg, s.c.) once a day for 14 days (SI vs. CI). In Experiment 1, the SI and CI groups were challenged with nicotine (0.5 mg/kg sc) on withdrawal days 1, 3 or 7 to determine time-dependent alterations in the nicotinic stimulation of locomotor activity. In experiment 2, SI and CI animals were treated with single daily injections of either saline or the non-selective (nAChR β2-preferring) antagonist mecamylamine (1.5 mg/kg i.p.) between withdrawal days 1 and 5 (SI-S, SI-M, CI-S or CI-M). On withdrawal day 7, all animals were acutely challenged with cocaine (7.5 mg/kg, i.p.), and their locomotor activity was measured to assess effects of mecamylamine treatment on cocaine sensitization. They were again challenged with cocaine on day 14 of cocaine withdrawal (9 days after the last mecamylamine treatment).

Results: Rats pretreated with cocaine injections (CI) showed enhanced locomotor responsivity to acute nicotine challenge compared to SI controls on days 3 and 7 but not day 1 of withdrawal. These results indicate progressive increases in nicotinic sensitivity during the first week of cocaine withdrawal. Consistent with the hypothesis that such increases in nicotinic receptor sensitivity may play a role in the consolidation of cocaine sensitization, the non-selective nicotinic receptor antagonist mecamylamine attenuated cocaine sensitization when administered during the first 5 days of cocaine withdrawal. Thus, while the CI-S group showed increased locomotor activity after cocaine challenge (i.e., locomotor sensitization), the CI-M group was statistically indistinguishable from the SI-S or SI-M group on both withdrawal days 7 and 14.

Discussion: Treatment with a nicotinic receptor (nAChRβ2-preferring) antagonist during the early cocaine withdrawal period may facilitate long-term abstinence by preventing consolidation of dysfunctional changes in critical "addiction pathways" in the brain.

Disclosure: S. Szabo: None. **J. Fowler:** None. **K. Bhatia:** None. **T. Lee:** None.

216. Preclinical Evidence that Alcohol Intake can be Suppressed by an H3 Receptor Antagonist

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Background: Modulation of the histamine system is not commonly listed in the emerging strategies for the treatment of alcoholism despite the fact that several studies have suggested a role for brain histamine in drug reward and addiction. Emerging preclinical data suggest that H3 receptor antagonists might be useful for the treatment of alcoholism. Histamine levels are higher in alcohol-preferring than in alcohol-nonpreferring rat brains, and expression of histamine H3 receptor (H3R) is different in key areas for addictive behavior. Mice lacking the H3 receptor showed lower alcohol preference ratios in a two bottle choice compared to control mice. The present study characterized the effects of a selective and brain penetrant H3 receptor antagonist (JNJ-39220675) on ethanol self-administration in ethanol-dependent and nondependent rats. The compound was also tested in a preclinical model of binge-like alcohol drinking. In addition, the effect of JNJ-39220675 on alcohol-induced dopamine release in the nucleus accumbens was tested in freely moving rats.
Methods: Ethanol self-administration experiments were conducted in operant chambers. Ethanol dependence was induced by intermittent exposure to ethanol vapors for 4 weeks and rats were subsequently tested for ethanol and water self-administration 6 hours into acute withdrawal. For the binge-like alcohol drinking experiments, rats were divided into alcohol binge drinkers and supersac controls. Following baseline training, rats were injected with JNJ-39220675 before two-bottle choice test sessions. The effect of JNJ-39220675 on alcohol-induced dopamine release in the nucleus accumbens was tested in freely moving ratsusing *in vivo* microdialysis.
Results: JNJ-39220675 (0.3, 3 and 10 mg/kg s.c.) significantly decreased ethanol self-administration in withdrawn, dependent rats and nondependent rats. The compound did not have a significant effect on water intake in withdrawn, dependent rats and nondependent rats. In the binge-like alcohol drinking model, JNJ-39220675 was found to produce a dose dependent reduction of intake of sweetened alcohol solution (10% w/v). In a separate cohort of control rats trained to drink just a sweetened solution, the compound did not produce any significant effect thus JNJ-39220675 had no "non-specific" effects on fluid intake. The H3 antagonist by itself at 3 mg/kg s.c. (in the absence of alcohol) had no effect on dopamine release in the nucleus accumbens. In addition, alcohol-induced dopamine release in the nucleus accumbens was not blocked by JNJ-39220675 indicating that JNJ-39220675 did not reduce the reinforcing effects of alcohol by changing dopamine release in the brain.
Discussion: These data strongly suggest that selective antagonism of the H3 receptor might be a potential target for the treatment of alcoholism in the near future. With a number of H3 antagonists having been disclosed as being advanced into clinical trials, it is now possible that this concept could be tested in the clinic.
Disclosure: T. Lovenberg: Part 1- 3, 5: Employee of Johnson & Johnson, parent company of Janssen Pharmaceutical R&D, LLC. Part 4: Work was funded by Janssen Pharmaceutical R&D, LLC. **P. Bonaventure:** Part 1- 3, 5: Employee of Johnson & Johnson, parent company of Janssen Pharmaceutical R&D, LLC. Part 4: Work was funded by Janssen Pharmaceutical R&D, LLC. **L. Aluisio:** Part 1- 3, 5: Employee of Johnson & Johnson, parent company of Janssen Pharmaceutical R&D, LLC. Part 4: Work was funded by Janssen Pharmaceutical R&D, LLC. **I. Fraser:** Part 1- 3, 5: Employee of Johnson & Johnson, parent company of Janssen Pharmaceutical R&D, LLC. Part 4: Work was funded by Janssen Pharmaceutical R&D, LLC. **M. Azar:** Part 1: I run the preclinical CRO that conducted some of the studies, Part 3: I run the preclinical CRO that conducted some of the studies, Part 5: Behavioral Pharma Inc. **M. Brennan:** Part 5: The Scripps Research Institute. **G. Koob:** Part 1: Addex Pharmaceuticals Alkermes Arkeo Pharmaceuticals Casa Palmera Embera NeuroTherapeutics GlaxoSmithKline Lilly Psychogenics.

217. Evidence for Significant White Matter Alterations following Chronic Cocaine Exposure in a Nonhuman Primate Model of Drug Self-Administration

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Background: Recent neuroimaging studies have shown that cocaine users have significant disruptions in functional brain connectivity suggesting deficits in white matter pathways. In addition, studies using DTI report abnormalities in white matter integrity in similar populations. The goal of these studies is to characterize the influence of cocaine exposure on white matter using a nonhuman primate model of cocaine self-administration in which carefully controlled experimental conditions can avoid the confounds inherent in studies of human substance abusers. Given that the induction of microglia to an activated state is a hallmark of disorders in which white matter is compromised, we examined activation of microglia in white matter at the level of the prefrontal cortex and striatum. In addition, we measured myelin-associated proteins in adjacent tissue.
Methods: Rhesus monkeys (n = 4) self-administered cocaine (0.3 mg/kg/inj, 30 reinforcers per session) for 15 months (total intake ~2750 mg/kg). Control monkeys (n = 4) responded for food reinforcement under an identical schedule. Following the final session monkeys were sacrificed and brains were processed for autoradiography using [3H]PK11195, a selective ligand for the peripheral benzodiazepine receptor, a marker for activated microglia in the CNS. White matter tissue at the level of the precommissural striatum was processed for immunoblotting analysis of two isoforms of proteolipid protein (PLP), myelin basic protein (MBP), and myelin-associated glycoprotein (MAG).
Results: Following long-term cocaine self-administration there was a widespread increase in [^{3}H]PK11195 binding, compared to controls, in white matter, that was most prominent at the level of the precommissural striatum. Elevations were observed in white matter tracts including the cingulum bundle, internal capsule, and corpus callosum, as well white matter extending to dorso- and ventrolateral cortex. These elevations were restricted to white matter bundles and were not present in subcortical gray matter. Prolonged exposure to cocaine also resulted in significantly lower levels of MBP, and both isoforms of PLP when compared to the levels of controls. No differences in the levels of MAG were noted.
Discussion: These data demonstrate significant alterations in the composition or structural integrity of myelin following chronic cocaine exposure in a nonhuman primate model of cocaine self-administration. Furthermore, chronic cocaine exposure resulted in an inflammatory response which may contribute to, or exacerbate, white matter abnormalities. These deficits may be the basis of the abnormalities in white matter and connectivity observed in human cocaine abusers. They also provide further evidence that the effects of chronic exposure to cocaine reach beyond its local effects in the dopamine system to impact more widespread targets, including non-neuronal cell populations.
Disclosure: L. Porrino: None. **H. Smith:** None. **S. Wang:** None. **T. Beveridge:** None. **M. Nader:** None.

218. Self-Administration of Compounds with Functional Selectivity at Gamma-Aminobutyric Acid Type A Receptor Subtypes in Midazolam- but not Cocaine-Experienced Monkeys

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Background: According to current views of the neurobiological basis of benzodiazepine (BZ) addiction, compounds without activity at alpha1 subunit-containing $GABA_A$ receptors should lack abuse potential due to an inability to facilitate activation of dopamine neurons in the ventral tegmental area.
Methods: To evaluate this hypothesis, rhesus monkeys were trained to self-administer intravenous (i.v.) injections of either midazolam (a short-acting benzodiazepine with non-selective agonist efficacy at alpha1, 2, 3 and 5 subunit-containing $GABA_A$ receptors) or cocaine (monoamine uptake blocker) under a progressive-ratio schedule, in which response requirements doubled during the course of a session. We evaluated the reinforcing effects of classical benzodiazepines, midazolam and lorazepam, as well as MRK-696 which has weak partial agonist efficacy at each of the four subtypes. Three alpha1-sparing compounds were evaluated: TPA023B and MRK-623 (zero intrinsic efficacy at alpha1, partial agonist at alpha2, 3, and 5), and TP003 (zero efficacy at alpha1, low partial agonist efficacy at alpha2 and alpha5, full efficacy at alpha3).
Results: Both midazolam and lorazepam maintained self-administration at relatively high levels under both baseline conditions, whereas MRK-696 maintained significant self-administration at relatively low levels under both baseline conditions. The alpha1-sparing compounds, however, had a unique profile of self-administration: All three were self-administered under midazolam baseline conditions, but none were self-administered under cocaine baseline conditions. Under the midazolam baseline, all three compounds maintained break points (i.e., highest response requirement obtained) lower than either midazolam or lorazepam.
Discussion: The hypothesis that alpha1-sparing compounds lack abuse potential is not generally applicable. Rather, alpha1 activity is important under a stimulant (i.e. cocaine) baseline, whereas all $GABA_A$ receptor agonists have reinforcing effects under a BZ baseline irrespective of their intrinsic efficacy profiles. Moreover, the results with TP003 suggest that only alpha3 receptor activity is necessary to maintain self administration, although relatively high efficacy at alpha3 receptors does not necessarily result in reinforcing strength equal to non-selective full agonists.
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219. Deficits in Ventral Prefrontal Cortex Group1 Metabotropic Glutamate Receptor Function Mediate Resistance to Extinction during Protracted Withdrawal from an Extensive History of Cocaine Self-Administration

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Background: Anomalies in excitatory transmission with prefrontal cortex (PFC) are theorized to contribute to poor inhibitory control over behavior, behavioral inflexibility, as well as drug craving, in addiction. The molecular mechanisms underpinning drug-induced PFC dysfunction are largely unknown. As Group I metabotropic glutamate receptors (mGluRs) are critical for drug reinforcement/reward, as well as drug-related learning, we examined for changes in the expression of mGluR1/5 within PFC subregions produced during protracted withdrawal from an extensive history of cocaine self-administration and then tested the functional relevance of observed changes for cocaine craving and extinction learning using behavioral pharmacological approaches.
Methods: Immunoblotting was conducted on ventral PFC (vPFC) and dorsal PFC (dPFC) tissue derived from rats trained to self-administer cocaine (0.25 mg/infusion) during 10 daily 6-hr sessions that were subjected to a 2-hr test for cue-reinforced behavior, in the absence of any further cocaine/saline delivery, at either 3 or 30 days withdrawal. Control animals received daily 1-hr or 6-hr training to lever-press for saline, and were sacrificed also following cue testing. Follow-up behavioral studies examined the effects of intra-PFC infusions of mGluR5 antagonists (3 mg/side MPEP and MTEP), mGluR1 antagonist (15 ng/side JNJ 16259685) or an mGluR1/5 agonist (27.5 ng/side DHPG) on cue-reinforced behavior and on the extinction of behavior with subsequent testing.
Results: Animals with a history of cocaine self-administration exhibited time-dependent: increases in cocaine craving and impairments in extinction learning (for both measures: IV X Withdrawal ANOVA, $p<0.05$). These behavioral phenomena were related to a time-dependent reduction in vPFC Group 1 mGluR expression (ANOVA, $p<0.05$). While a history of cocaine self-administration elevated dPFC levels of mGluR1/5, this increase did not vary as a function of withdrawal. Mimicking the vPFC cocaine effect via intra-vPFC infusion of antagonists at 3 days withdrawal produced no acute effect on cue-reinforced behavior in either saline or cocaine self-administering animals (IC X IV ANOVA: $p>0.05$), but impaired extinction learning manifested upon subsequent testing only in animals with cocaine experience (IC X IV ANOVA, $p<0.05$). Stimulating mGluR1/5 via intra-vPFC infusions of DHPG at 30 days withdrawal also produced no acute effects on cocaine craving (one-way ANOVA, $p>0.05$), but facilitated extinction learning as manifested on a subsequent test for craving in cocaine-experienced animals only (IC X IV ANOVA, $p<0.05$).
Discussion: The present report provides *in vivo* validation of an important role for vPFC Group1 mGluRs in learning to suppress cocaine-seeking behavior during cocaine abstinence by showing that the site-directed pharmacological manipulation of both mGluR1 and mGluR5 function within the vPFC bi-directionally affects extinction learning in animals with an extensive history of cocaine self-administration. Taken altogether, these results support the hypothesis that a time-dependent reduction in vPFC Group I mGluR function is a neural adaptation produced during withdrawal from an extensive history of cocaine self-administration that impairs the capacity of an addicted individual to learn new stimulus-response contingences during protracted drug abstinence. If relevant to humans, such findings implicate a progressive decrease in vPFC mGluR1/5 function during drug abstinence as a molecular cordon to recovery, which may be best overcome using receptor agonist treatment strategies.
Disclosure: O. Ben-Shahar: Part 2: UCSB. **A. Sacramento:** Part 2: UCSB. **B. Miller:** Part 2: UCSB. **S. Webb:** Part 2: UCSB NIDA. **M. Wroten:** Part 2: UCSB. **A. Caruana:** None. **E. Gordon:** None. **K. Ploense:** Part 2: UCSB. **J. Ditzhazy:** None. **T. Kippin:** Part 2: UCSB. **K. Szumlinski:** Part 2: UCSB.

220. The Novel Neuropeptide S - Receptor Antagonist, NCGC00185684, decreases Alcohol Self-Administration in Rats by Suppressing Motivation for Alcohol

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Background: The prevalence of lifetime alcohol abuse in the US was about 18% in 2007 (Hazin *et al.*, 2007). However, only a limited number of pharmacotherapies exist. Neuropeptides and their receptors may offer putative targets for treatment development for alcohol abuse. Recently, attention has focused on neuropeptide S (NPS) and its receptor (NPS-R). The NPS-R is G-protein coupled and was initially described by Sato *et al.*, (2002), and deorphanized by Xu *et al.*, (2004). Central administration of NPS promotes hyper-locomotion and arousal (Xu *et al.*, 2004), and suppresses food-intake (Fedeli *et al.*, 2009). Furthermore, administration of NPS into the lateral hypothalamus increases cue-induced alcohol-seeking (Cannella *et al.*, 2009). The NPS-R is expressed in brain areas associated with drug addiction and reward (Xu *et al.*, 2007). Here, we present data using a novel, peripherally available NPS-R antagonist, NCGC00185684, in models of alcohol intake, motivation to take alcohol, and reinstatement of alcohol seeking.
Methods: Subjects: Male Wistar rats (300 g at start of experiment, Charles-River, PA) were used. Animals were housed on a reversed light cycle (on at 8:30 pm/off at 8:30 am) in a controlled environment with food and water available *ad lib*. All procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals (1998) and approved by the NIAAA ACUC. Drug: The NPS-receptor antagonist NCGC00185684 was prepared by Drs Patnaik and Marugan at the NIH Chemical Genomics Center, NHGRI, NIH, Bethesda, MD, and dissolved in 10% Solutol, 10 % N,N-dimethylacetamide, and 80% 10 mM PBS, pH 7.4. Animals were injected 2 hours prior to testing. Food-Intake Studies: Animals equipped with icv guides were trained to consume a sweetened diet for 1 hr per day. Following establishment of baseline, animals were treated with vehicle, NPS, or NPS plus NCGC00185684 in a latin square design. Operant alcohol self-administration: Animals were trained to self- administer a 10% alcohol-solution (FR-3 schedule) in sound-attenuated self-administration boxes (MedAssociates, VT).
Cue-and stress induced reinstatement: During self-administration training animals were presented with orange cue scent. Following extinction sessions ($n = 15$) animals in the cue-induced reinstatement group were presented with odor cue prior to a self-administration session while animals in the stress- induced reinstatement group were given a 15 minute intermittent foot-shock session immediately prior to reinstatement testing.
Results: Alcohol Self-Administration: Treatment with the NPS-R antagonist NCGC00185684 at a dose that did not affect locomotor activity (1 mg/kg) significantly decreased responding at the alcohol-associated lever ($F[3, 45] = 7.95$, $p = 0.0002$, Newman-Keul *post hoc*: $p < 0.005$ 1 mg/kg vs. vehicle). Responding at the inactive lever was unaffected by treatment (data not shown). Cue-and stress- induced reinstatement: Both cue ($F[1,14] = 21.20$, $p = 0.0004$) and stress ($F[1,15] = 34.13$, $p < 0.0001$) successfully reinstated responding at the previously alcohol-paired lever. However, treatment with NCGC00185684 1 mg/kg did not significantly affect reinstatement-responding (Cue: $F[1, 14] = 0.002$, $p = 0.97$; Stress: $F[1,15] = 1.24$, $p = 0.28$). Progressive ratio breakpoint: Treatment with NCGC00185684 (1 mg/kg) significantly decreased the progressive ratio breakpoint ($t = 2.42$, $p = 0.02$, $n = 7$-8 per group). Food-Intake study: Icv administration of NPS (10 microgram) induced a reduction in intake of a sweetened diet (Treatment effect: $F[2, 19] = 3.6402$, $p = .04588$ vs. vehicle). Pre-treatment with NCGC00185684 (icv, 10 microgram) prior to NPS administration reversed this anorectic effect of NPS (Newman-Keul post-hoc: $p = 0.78$ ((NPS + NCGC00185684) vs. vehicle) and $p = 0.047$ ((NPS + NCGC00185684) vs. NPS alone).
Discussion: Here, we present data supporting a role for NPS and its receptor in regulation of alcohol intake related behaviors. The NPS-R antagonist NCGC00185684 reduced alcohol self-administration at a dose not affecting locomotor behavior, while leaving both cue- and stress-induced reinstatement of alcohol-seeking unaffected. Treatment with NCGC00185684 decreased motivation to take alcohol as indicated by the decreased breakpoint in progressive ration testing. The specificity of the antagonist for the NPS-receptor and in regulating behaviors mediated by the NPS-R, was indicated by the ability of icv pre-treatment with NCGC00185684 to reverse the reduction in intake of a sweetened diet induced by NPS. In conclusion, we show the NPS-R to be an interesting target for development of pharmacological treatments of alcohol dependence.
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221. Orexin mediates Yohimbine Actions in BNST and Impaired Extinction of Cocaine Place Preference through a Norepinephrine-Independent Process

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Background: The alpha2 adrenergic receptor (a_2-AR) antagonist yohimbine is a widely used tool for the study of anxiogenesis and stress-induced drug-seeking behavior. We previously demonstrated that yohimbine produces both a paradoxical depression of excitatory transmission in the bed nucleus of the stria terminalis (BNST) along with an impairment of extinction of cocaine conditioned place preference (cocaine CPP) that are independent of (a_2-AR) signaling. The target of yohimbine that mediates these actions, however, is unknown.
Methods: We utilized whole-cell patch clamp and field potential recordings in the BNST*ex vivo* to examine the effects of yohimbine (30-50 uM), orexin A (100 nM), orexin 1 receptor (Ox1R) antagonist (SB-334867; 2-5 uM), orexin 2 receptor (Ox2R) antagonist (JNJ-10397049), and a novel dual OxR antagonist MTBDQ (1uM), and the norephinephrine transport blocker reboxetine (100 nM) on excitatory transmission in wild-type and prepro-orexin knockout mice (Ox-KO). The ability of yohimbine to directly activate Ox1R was investigated utilizing an Ox1R expressing stable cell line. To examine the potential behavioral implications of these findings, the ability of OxR antagonists to alter yohimbine impairment of cocaine CPP extinction was investigated.
Results: Yohimbine-induced depression of excitatory transmission in the BNST is blocked by two distinct OxR antagonists, absent in Ox-KO mice, and mimicked by exogenous orexin A application but not blockade of norepinephrine uptake. Moreover, we show that the action of yohimbine is not through direct activation of Ox1R, suggesting yohimbine facilitates orexin release. Behaviorally, we find that yohimbine-induced impairment of cocaine CPP extinction is also blocked by an Ox1R antagonist.
Discussion: These data describe a major new mechanism for orexin action on excitatory anxiety circuits, and show that significant behavioral actions of yohimbine may be directly dependent upon orexin signaling and independent of norepinephrine.

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222. Effects of Ethanol on Endocannabinoid Modulation of Up-States in Prefrontal Cortex

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Background: Alcohol (ethanol) is one of the most widely used substances in the world and its manufacture and sale are of major economic importance. However, alcohol consumption is also associated with heavy costs in terms of lost productivity, disruption of family and social interactions and disease and death. Factors that underlie uncontrolled drinking are complex but may involve disruption of brain areas that normally prevent individuals from engaging in risky behaviors and impulsive decision-making. Of these regions, areas of the prefrontal cortex are thought to be especially important and lesions of these areas produce cognitive and behavioral deficits that resemble those observed in human alcoholics. Recent studies in this laboratory have focused on understanding the actions of ethanol on PFC function and we have developed and utilized *in vitro* models that recreate physiologically relevant patterns of neuronal activity observed *in vivo*. These approaches allow for a detailed examination of the processes that underlie normal activity and how these are disrupted by ethanol. Results from these studies suggest that inhibition of NMDA receptors may be a key feature of the initial effects of ethanol on brain function. More recent studies have focused on understanding how modulators of neuronal excitability such as endocannabinoids (ECs) are impacted by alcohol. In this study, we characterize the effects of cannabinoid signaling on activity in PFC neurons and investigate how this system responds to a chronic exposure to ethanol.
Methods: Slice co-cultures were prepared from postnatal day 1-5 C57Bl6/J mouse pups. Coronal sections containing prefrontal cortex (including anterior cingulate, prelimbic and infralimbic cortex) were oriented adjacent to one another on a millicell insert in a 6-well culture dish. Cell culture dishes were kept in a humidified incubator equilibrated with 5% CO_2 and were maintained for 15-40 days with media changes every 2-3 days. Up-states were monitored in deep-layer pyramidal neurons located in the prelimbic portion of the slice culture by whole-cell patch-clamp electrophysiology. In some studies, changes in intracellular calcium were monitored using a genetically encoded calcium sensor (GCaMP3.3) delivered with an AAV virus. To determine the effects of chronically administered ethanol, cultures were incubated in media containing 44 mM ethanol ($\sim$0.2% BEC) for 10 days and then allowed a 4-day withdrawal period before analysis.
Results: Deep-layer pyramidal neurons in organotypic cultures of the prefrontal cortex displayed spontaneous and stimulus-evoked changes in membrane potential that persisted for several seconds. This activity was characterized as a rapid transition in membrane potential from $\sim$-72 mV during the down-state to $\sim$-55 mV during the up-state. This change in membrane potential was accompanied by bursts of action potentials riding on top of the up-state. GCaMP3.3 measurements revealed that up-states induced robust and simultaneous increases in intracellular calcium in most neurons in the culture. Applied acutely, ethanol inhibited up-states at concentrations associated with behavioral impairment. Application of the CB1 receptor agonist WIN-55,212-2 or inhibitors of EC degradation increased up-state amplitude but had no effect on the duration of up-states. In contrast, the CB1 inverse agonists AM-251 or AM-281 increased the frequency of GABA IPSCs and decreased up-state duration without affecting amplitude. While up-states were observed in slices made from CB1 knock-out mice, they were longer than those in wild-type cultures and were unaffected by WIN. Cultures exposed to 44 mM ethanol for 10 days and then withdrawn for 4 days showed enhanced up-state duration and reduced levels of CB1 protein. This was accompanied by a blunting of the ability of WIN to enhance up-state amplitude suggesting a down-regulation in the functional status of CB1 receptors.
Discussion: Previous studies suggest that ECs are involved in a wide variety of processes including regulation of various emotional and affective states. In the prefrontal cortex, ECs may regulate the activity of neurons involved in cognitive flexibility, a behavior that is impacted by chronic alcohol drinking. The results of this study show that ECs modulate up-state dynamics in a slice culture model of the prefrontal cortex and that exposure to and withdrawal from ethanol may disrupt this regulation. Alterations in EC signaling by ethanol may contribute to impairments in cognitive ability and risk assessment that are associated with alcohol use and abuse.
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Disclosure: J. Woodward: None. **M. Pava:** None.

223. Inverted-U Relationship between Cortical Oscillations and Dopamine: EEG and Computational Studies

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Background: It has been hypothesized that cortical function may have an inverted-U shaped dependence on dopamine (DA) levels. There is evidence that schizophrenia (SZ) is associated with decreased cortical DA levels, while healthy control subjects (HC) may already have optimal DA levels. Thus, administration of pharmacologic agents that increase cortical DA may improve cortical function in SZ subjects while impairing cortical function in HC. To test this putative relationship, we examined the effect of single-dose dextro-amphetamine (d-Amph) on cortical responses in an auditory click-train paradigm and conducted computational studies to explore potential physiologic mechanisms by which such relationships may be mediated.
Methods: 12 HC and 12 SZ subjects who participated in a double-blind, cross-over, placebo (PBO)-controlled study of single-dose d-Amph administration. After medication administration, subjects had EEG measured during auditory click trains presented at 20, 30 and 40 Hz. The spectral power of the steady-state auditory evoked potential was determined with wavelet analyses. Computational simulations in biophysically realistic networks were conducted to model the effects of dopamine on oscillatory rhythms through excitability of fast-spiking interneurons.
Results: For the PBO condition, SZ showed lower gamma (40 Hz) power compared to HC, replicating previous studies. However, SZ showed improvements in gamma power with d-Amph administration compared to placebo, while HC showed less gamma power with d-Amph compared with PBO. Computational simulations demonstrated that parametric increases in the excitability of fast-spiking interneurons could give rise to the inverted-U pattern of cortical oscillations.
Discussion: Our results provide evidence that increasing cortical dopamine may enhance cortical activity in schizophrenia subjects while impairing cortical responses in healthy subjects, consistent with an inverted-U shaped relationship between cortical activation and dopamine levels. Our computational findings offer a basic

mechanism by which dopamine's effects at the single unit level in fast-spiking interneurons can give rise to such an inverted-U relationship at the neural network level. The findings of increased gamma synchrony with amphetamine administration in schizophrenia demonstrates a possible mechanism by which dopamine's effects on cortical function are mediated, and in turn, offers a possible novel therapeutic mechanism for remediating disturbed cortical oscillations in schizophrenia and other neuropsychiatric disorders.
Disclosure: R. Cho: None. **C. Walker:** None. **K. Komek:** None. **G. Ermentrout:** None. **R. Verbiest:** None.

224. Does the NMDA-Receptor Antagonist, Ketamine, Mimic the Pattern of EEG Gamma Oscillation Abnormalities Observed in Schizophrenia? A Test of the NMDA-Receptor Hypofunction Model

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Background: Gamma oscillations have been implicated in the coordination of neural activity among distributed networks of neuronal assemblies, and in neurocognitive functions including sensory registration, perceptual binding, and cognitive control. Glutamatergic NMDA-receptors are known to modulate the microcircuitry subserving gamma oscillations in the brain. EEG abnormalities in the gamma band have been repeatedly documented in schizophrenia. Furthermore, converging lines of evidence implicate glutamatergic NMDA receptor hypofunction as a pathophysiological mechanism in schizophrenia, mediating both clinical symptoms and neurocognitive impairments. At subanesthetic doses, the non-competitive NMDA receptor antagonist, ketamine, induces a spectrum of transient *schizophrenia-like effects* in healthy human subjects including positive and negative symptoms, cognitive impairments and electrophysiological changes. In order to examine the plausibility that NMDA-receptor hypofunction mediates abnormal gamma band activity in schizophrenia, we asked whether ketamine administered to healthy controls results in the same profile of gamma band abnormalities as seen in schizophrenia.
Methods: EEG auditory steady-state responses (ASSR), recorded during a steady state driving paradigm involving 500 ms click trains presented at frequencies of 20, 30, and 40 Hz, were obtained from: (a) 47 patients with schizophrenia or schizoaffective disorder (SZ) and 50 age- and gender-matched healthy controls; and (b) 26 healthy controls who received placebo (saline) or intravenous ketamine on two separate test days in a randomized double-blind cross-over design. Baseline gamma power (30-50 Hz) preceding all of the click trains (-200 to -100 ms) and gamma phase locking factor (PLF) during only the 40 Hz click train (200-500 ms), were calculated using a Morlet Wavelet decomposition of single trials.
Results: Consistent with prior schizophrenia studies, there was (1) a significant decrease in 40 Hz PLF and (2) a significant increase in baseline gamma power in SZ. During ketamine administration, subjects exhibited (1) no change in 40 Hz PLF but (2) significantly enhanced baseline gamma power. The latter result is consistent with previous animal and human studies of NMDA-receptor blockade.
Discussion: These results suggest that NMDA-receptor blockade by ketamine enhances intrinsic baseline gamma power (i.e., "gamma noise") while not affecting the 40 Hz ASSR. In contrast, patients with schizophrenia have increases in gamma baseline power and significant 40 Hz steady-state PLF reductions relative to control subjects. The comparison of schizophrenia with acute NMDA receptor antagonist administration suggests that NMDA receptor hypofunction may mediate the increased magnitude of intrinsic gamma oscillations in schizophrenia, but it is unlikely to be the source of their deficient synchronization of extrinsically driven gamma oscillations.
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225. Homeostasis and Quantitative Sleep EEG in Alcohol Dependent Adults

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Background: Alcohol dependence has been associated with a significant reduction in slow-wave EEG activity (SWA) during sleep. However, it is unclear whether reduced SWA reflects impaired regulation of sleep homeostasis, or is due to hyperarousal and increased fast-frequency beta EEG. The present study tested these hypotheses in adults with alcohol dependence, who were abstinent at the time of study. Both baseline sleep and sleep after a homeostatic challenge were assessed.
Methods: 48 alcohol-dependent (AD) adults (39 men, 9 women) who were abstinent for at least 3 weeks and 16 healthy control (HC) adults (13 men, 3 women), 21-55 years of age, participated in study. Each participant maintained an 11pm-6am schedule for the week prior to the lab study (verified by actigraphy and sleep diary data) followed by 3 consecutive nights of polysomnography in the lab. Night 1 served as laboratory adaptation. Baseline sleep EEG measures were collected on night 2. The third night served as a challenge to sleep homeostasis, delaying bedtime to 2 am but still allowing 7 hours of available sleep time. Power spectral analysis (PSA) quantified all-night power in each of beta (16-32 Hz), sigma (12-15.9 Hz), alpha (8-11.9 Hz), theta (4-7.9 Hz) and delta (0.5-3.9 Hz) EEG frequencies in each hour of sleep. Both absolute power and relative total power were computed in each frequency band and compared between AD and HC groups. SWA, delta exclusively within episodes of NREM sleep, was also computed on the baseline and sleep delay nights. %SWA was computed as an index of homeostatic response, expressing SWA within each successive NREM sleep period on the baseline night relative to SWA within each successive NREM sleep period after the sleep delay.
Results: The AD group had significantly less absolute SWA power and showed a blunted SWA response to challenge. Those in the AD group showed the slowest decline in SWA over NREM sleep time, with a decay rate that was outside the 95 % confidence intervals that of HCs. Relative, but not absolute, beta power was 4 % higher in the AD group, but the effect size was small. By contrast, SWA power and response to the sleep challenge was 30% lower in the Ads compared to HCs. None of the other EEG frequencies differed between groups.
Discussion: Impaired homeostatic sleep regulation, not hyperarousal, underlies sleep disturbances in alcohol dependence. These findings may reflect impaired neuronal recovery during sleep. Evidence for hyperarousal during sleep in the alcohol-dependent group was weak. These findings may be of clinical relevance in developing behaviorial interventions to improve sleep in alcohol dependence.
Disclosure: R. Armitage: Part 1: Consultant - Eisai Inc. **R. Hoffmann:** None. **J. Arnedt:** None. **D. Conroy:** None. **K. Brower:** None.

226. The Effect of Cocaine in Higher Order Local Circuitry as Revel by Optogenetics and Pharmacological Methods- Orchestrate and Disorganize

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Background: Drugs of abuse have an enormous social and individual cost by degrading individual cognitive abilities, emotional stance and social behavior and bonds. Their impact cannot be overestimated. Therefore a good amount of research is directed towards understanding the mechanisms of addiction. Most of the research in addiction, so far has been aimed at the reward circuitry (limbic system), and less attention has been paid to higher processing areas, although these areas are responsible too for the catastrophic effects of addiction. In order to understand the influence of cocaine on higher order cognitive abilities through the local circuitry of the prefrontal cortex, we use a knock-in mouse model together with optogenetics and electrophysiology Prefrontal cortex integrates higher and lower order activity into meaningful behavioral patterns on the short and the long run (i.e. planning). In order to achieve this role of integrator, it has to have a highly orchestrated and well timed way of processing information. From the literature it seems that different frequency neural oscillations play an important role in this integrative machinery. Besides the system-wide oscillations (theta), local oscillations (gamma) are of utmost importance. They are generated by local oscillators, which are networks in which pyramidal and interneurons work together in a well ordered manner. In these oscillators parvalbumine-positive, GABA-ergic (fast spiking) interneurons seem to have an orchestrating role. These interneurons receive dopaminergic innervation, so they are a potential target of the effects of cocaine. Since they are in the center of the integrating mechanism, their role in the addiction-related cognitive deficits may be central. Our study aims to investigate this role.
Methods: Parvalbumin Cre knock-in adult male mice (B6;129P2-Pvalb $^{tm1(Cre)Arbr/J}$ Jackson Laboratory (Bar Harbor, ME, USA) were injected with AAV virus(AAV-ChR2-YFP, Univ. of Pennsylvania) containing channelrodopsin genes so that around the injection site (prefrontal cortex: + 2 mm anterior, 0.5 mm lateral, 1 mm ventral from bregma) parvalbumine-positive interneurons express ChR2. We recorded local field potentials, multiunit and single unit activity from the anaesthetized animals, whilst stimulating with laser light (473 nm laser, DPSS Laser System, OEM Laser Systems Inc, East Lansing, MI, USA) through custom made optrodes. We applied cocaine (IP, 15 mg/kg), and dopamine-receptor antagonists (sulpiride, 15 mg/kg, IP; SCH23390, 1 mg/kg, IP), to study the pharmacology of the network activity at the single cell, multiunit and local field evoked potential level. Our set of analysis tools included t-tests, anovas, power-spectrum analysis and Levene's test for the homogeneity of variance.
Results: The most robust result was a significant decrease in the variance of the evoked responses following an acute ip injection of cocaine. Both dopamine antagonists brought back the variance close to the baseline. At the single cell level, in the case of the parvalbumin-positive interneurons the effect of cocaine was mixed. In 40 % of the neurons, acute cocaine injection increased the average firing rate whereas in 60% of the interneurons acute cocaine injections did not change the firing rate. With respect of the evoked gamma potentials we also observed mixed results: in 47% of the cases (8/17 animals) acute cocaine injection did not alter the power of gamma rhythms, in 35% of the recordings (6/17 animals) acute cocaine injection increased the power of gamma rhythms and in 21% (4/17) of the cases, cocaine deceased the power of evoked gamma rhythms.
Discussion: Our preliminary experiments show that acute cocaine administration is affecting the intrinsic activity of some fast spiking, PV + cortical interneurons. Since the fast spiking PV + interneurons underlie the generation of gamma rhythms, the single cell effects may be translating in changes in gamma power, and since gamma rhythms are thought to be the cellular correlates of cognitive functions, the effect of cocaine in fast spiking, PV + cells may elicit cognitive changes partially responsive for the engagement and perseverance in incorrect decision making.
Disclosure: T. Tompa: None. **A. Lavin:** None.

227. Activation of Ventral Tegmental Area GABAergic Neurons disrupts Reward Consumption

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Background: The Ventral Tegmental Area (VTA) is a heterogeneous brain structure containing neuronal populations that are essential for the expression of motivated behaviors related to addiction and other neuropsychiatric illnesses. The VTA contains a mixture of dopaminergic (DAergic) (~65%), GABAergic (~30%), and glutamatergic neurons (~ 5%), which are thought to act in concert to orchestrate and regulate reward-seeking behavior. While these distinct populations of neurons may functionally interact with each to to guide behavioral responding, this has been difficult to test as methodologies for selectively modulating the activity of genetically defined VTA neuronal populations *in vivo* with high temporal precision was not possible. To circumvent this limitation we utilized optogenetic strategies to selectively target the light-gated cation channel, channelrhodopsin-2 (ChR2) into VTA GABAergic neurons to study how modulation of their activity altered reward-seeking behavior.
Methods: Adult mice expressing Cre recombinase under control of the endogenous vesicular GABA transporter promoter (*Vgat*-ires-Cre) were used as subjects. Mice were stereotactically injected into the VTA with adeno-associated virus capable of expressing ChR2-EYFP only in the presence of Cre recombinase. For mice used in behavioral experiments, optical fibers capable of delivering light to the VTA to activate VTA GABAergic neurons were also implanted. Following 3 – 4 weeks after surgery mice were either prepared for patch-clamp electrophysiological experiments or used in behavioral experiments. To examine how GABAergic activation modulated reward-related behaviors, we utilized a Pavlovian conditioning task where a 5 s tone/houselight stimulus reliably predicted the delivery of a sucrose reward. Following ~4 weeks of daily training in this task mice developed robust reward-seeking behavior as indicated by the number of licks that were made at the sucrose delivery receptacle both during cue presentation as well as following reward delivery. Following stable behavioral responding optogenetic activation of VTA GABAergic either timelocked to the reward-predictive cue or timelocked to reward consumption was performed.
Results: Expression of Chr2-EYFP was not detected in VTA DAergic neurons, but was widely observed in putative VTA GABAergic neurons. In fibers and processes originating from ChR2-EYFP expressing neurons, robust VGAT staining was also detected, suggesting that ChR2 expressing neurons were largely GABAergic. Whole cell recordings from fluorescently identified neurons revealed that application of the 470 nm light, capable of activating ChR2, led to a robust increase in firing rate up to ~60 Hz, which was sustained for the duration of the 5 s light pulse. Recordings from identified VTA DAergic neurons revealed that large amplitude inhibitory postsynaptic currents were detected in response to optical stimulation of GABAergic neurons. In addition, activation of GABAergic neurons significantly attenuated evoked firing of neighboring DAergic neurons suggesting that GABAergic neurons can functionally suppress DAergic activity in slices.

In vivo optogenetic activation of VTA GABAergic neurons timelocked to reward-predictive cue presentation did not alter either reward seeking during the cue presentation or consumption of the reward. However, optogenetic activation of VTA GABAergic neurons timelocked to reward delivery on each trial significantly disrupted reward consumption. Importantly, there was no difference in the total number of licks recorded during sessions with optogenetic activation of GABAergic neurons vs. session optogenetic activation was withheld.

Discussion: These data suggest that activation of VTA GABAergic neurons can disrupt reward consumption, but not cue-evoked reward seeking. Mechanistically, we hypothesize that local GABAergic activation may efficiently suppress DAergic activity when DAergic neurons are firing at sustained, tonic frequencies, but not at elevated firing rates associated with transient bursts of activity, such as those seen during reward-predictive cue presentation. Further, these studies demonstrate that modulation of genetically defined non-DAergic neuronal populations in the VTA can profoundly alter reward-related behaviors.

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