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Poster Session III
Wednesday, December 7, 2011 5:30 PM – 7:30 PM

1. BK Channel β1 Subunit Modulates Ethanol Drinking and Behavioral Adaptations to Chronic Ethanol Exposure

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Background: Alcohol abuse disorders have devastating health and societal consequences. Development of more efficient alcoholism treatments requires a better understanding of the molecular mechanisms mediating the intoxicating and motivational effects of ethanol. One of the well-established molecular targets of ethanol is the large conductance calcium-activated potassium (BK) channel. BK channels are highly expressed in the brain and play a key role in several aspects of neuronal physiology. Ethanol is a potent activator of BK channel gating, but *in vivo* evidence for a causal relationship between BK channel potentiation and ethanol's behavioral effects is scarce. Interestingly, association of the auxiliary β1 subunit with the pore-forming α subunit precludes ethanol-induced potentiation of BK currents *in vitro*. In the present study, we investigated how deficiency in BK β1 subunit affects ethanol intoxication, tolerance, dependence and drinking in mice.
Methods: Adult male BK β1 wild-type, heterozygous and knockout mice were trained and tested following the injection ofa low dose of ethanol (1.5 g/kg) in the accelerating rotarod assay of motor coordination. The hypnotic effect of a high dose of ethanol (4 g/kg) was measured in the loss-of-righting-reflex test, along with hypothermia. Mice were exposed to chronic intermittent ethanol vapor in inhalation chambers for 3 cycles of 8-h intoxication / 16-h withdrawal, and a time-course of handling-induced convulsions was conducted to evaluate dependence. Ethanol-induced ataxia, sedation, and hypothermia were measured approximately 26 h into withdrawal to assess the development of tolerance. An independent cohort of mice was subjected to a limited-access (2 h /day, starting 3 h into the dark phase) two-bottle choice model of voluntary ethanol drinking.
Results: We found that sensitivity to ethanol-induced ataxia, sedation and hypothermia was similar between BK β1 wild-type, heterozygous and knockout male mice. Chronic intermittent exposure to ethanol vapor produced tolerance to these effects in wild-type mice, but the extent of tolerance was reduced in knockout mice. Moreover, knockout and heterozygous mice experienced an earlier and more intense physical withdrawal syndrome than wild-type counterparts. We also found that knockout and heterozygous mice self-administered less ethanol than their wild-type littermates.
Discussion: These findings suggest that the β1 subunit may be recruited upon chronic intoxication to dampen ethanol-induced potentiation of BK currents, thereby minimizing behavioral responses to ethanol. Absence of the β1 subunit in knockout mice may, on the other hand, promote counter-adaptive changes downstream of BK channel overstimulation by ethanol and exacerbate withdrawal. Decreased drinking in BK β1 deficient mice suggests a key role of BK channels in the initial neuroadaptations to ethanol within the reward system.
Disclosure: C. Contet: None. **D. Le:** None. **A. Roberts:** None. **S. Treistman:** None. **G. Koob:** Part 1: Addex Pharmaceuticals Alkermes Arkeo Pharmaceuticals Casa Palmera Embera Neuro-Therapeutics GlaxoSmithKline Lilly Psychogenics.

2. Long-Term, 96-hour Methamphetamine Self-Administration in Rats: A Preclinical Model Of Human Methamphetamine Addiction

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Background: In 2009, the economic cost of methamphetamine use to society was estimated at $16.2 - $48.3 billion. Chronic methamphetamine use is also associated with crime, aggression, and violent, deviant and risky sexual behaviors. Human methamphetamine addicts typically follow a "binge-pattern" of use, with 3-15 days of continual drug use followed by a "crash" period of 1-3 days, often consisting of continuous sleep. They then usually repeat this cycle if methamphetamine is available. These binges of continuous methamphetamine use may underlie the development of many of the deviant behaviors observed in long-term methamphetamine addicts. However, in the preclinical laboratory, methamphetamine addiction is typically modeled using non-contingent drug injections and/or self-administration sessions under daily 2- or 6-hour access conditions. While these models have increased our understanding of both the molecular and behavioral intricacies of chronic methamphetamine exposure, they could be improved by more closely modeling human drug usage, which was the goal of these experiments. Of additional importance are potential gender differences associated with long-term methamphetamine use since there are few reports of the effects of methamphetamine on female rats, especially using a binge paradigm. Therefore, we studied the effects of 96-hour methamphetamine self-administration in both male and female rats.
Methods: Male and female adult Wistar rats were implanted with jugular catheters and allowed to recover from surgery. The rats were placed into operant chambers and trained to self-administer methamphetamine (0.06 mg/kg/infusion) for 96 consecutive hours. The rats had free access to water during the session and were fed once per day with standard lab chow to maintain weights. After 96 hours, the rats were returned to their home cages for 72 hours (withdrawal), which was followed by another 96-hour self-administration session. Blood samples were drawn before, during and after the self-administration sessions to monitor corticosterone, ACTH and testosterone. Estrous cycle fluctuations were also monitored in female rats.
Results: The patterns of self-administration evolved during the course of this experiment in both male and female rats. The rats initially self-administered more methamphetamine during their active cycles, maintaining regular active and inactive periods of drug intake. Over time, however, the rats displayed dramatic shifts in their circadian rhythms, self-administering more and more methamphetamine during their normally inactive periods compared to their active periods, often binging nonstop for up to 72 hours. Plasma corticosterone decreased in both male and female rats during methamphetamine exposure but increased back to baseline during withdrawal. Similar effects were seen with plasma ACTH in female rats. Male rats exhibited a similar effect on plasma testosterone, with decreases during methamphetamine exposure that increased back to baseline during withdrawal. No changes in plasma testosterone were seen in female rats. However, female rats displayed disruptions of the estrous cycle by the second exposure to the 96-hour methamphetamine sessions, which is consistent with human female menstrual cycle disruption reported with methamphetamine use. Finally, both male and female rats displayed increasingly violent, aggressive and self-injurious behaviors during the course of this experiment, all consistent with human methamphetamine use.

Discussion: These data suggest that our 96-hour binge model of methamphetamine self-administration may be useful as a novel preclinical model to more closely mimic human methamphetamine-taking behavior and perhaps to further contribute to elucidating gender differences in methamphetamine use and addiction.
Disclosure: N. Goeders: None. **G. Guerin:** None. **E. Cornett:** None.

3. Essential Role for Fragile-X Mental Retardation Protein (FMRP) in Normal Behavioral and Neuronal Morphological Adaptations following Repeated Cocaine Administration

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Background: Repeated exposure to cocaine produces sensitized locomotion and reward-associated learning (conditioned place preference; CPP) in rodents. We previously reported that expression of active myocyte enhancer factor 2 (MEF2), a transcription factor family that promotes activity-dependent excitatory synapse elimination, positively modulates both sensitized locomotion and CPP to repeated cocaine (Pulipparacharuvil *et al*., 2008, *Neuron*). In addition, we found that MEF2-dependent synapse elimination in hippocampal pyramidal neurons requires the RNA-binding protein, Fragile-X Mental Retardation Protein (FMRP) (Pfeiffer *et al*., 2010, *Neuron*). Functions of FMRP include dendritic transport and translational control of specific mRNA transcripts. Humans with Fragile X and mice lacking FMRP expression have increased dendritic spine density, possibly through defects in developmental synapse elimination. Given our previous findings, our goal is to determine the role of FMRP in drug-related behavioral and synaptic plasticity.
Methods: We examined behavioral, biochemical and morphological responses to cocaine exposure in *Fmr1* knockout and wild-type littermates and further explored the potential role of mGluR5 in FMRP-dependent cocaine behavioral adaptations using compound mutant mice. To determine if FMRP plays an active role in the adult brain, we utilized viral expression of CRE-recombinase in adult conditional *Fmr1* knockout mice.
Results: Consistent with our previous MEF2 findings, we observe that FMRP is required for normal cocaine-induced behavioral plasticity. While acute locomotor responses are not different, *Fmr1* null mice develop significant deficits in both sensitized locomotion and conditioned place preference to cocaine compared to wild-type (WT) littermates. However, unlike non-cocaine phenotypes previously reported, reduction of mGluR5 does not restore normal cocaine-induced phenotypes. Importantly, a significant portion of the *Fmr1* KO cocaine behavioral deficits is recapitulated by acute loss of FMRP in an adult reward-related brain region. These behavioral effects are accompanied by unique morphological responses of *Fmr1* knockout neurons to cocaine treatment.
Discussion: FMRP is required for normal cocaine-induced behavioral and morphological plasticity. Loss of normal drug-induced plasticity may relate to FMRP's role in mediating MEF2-dependent synapse elimination or to its role regulating long-term depression. In sum, our findings reveal a novel role for FMRP in the processes underlying alterations in the brain and behavior that occur in response to repeated cocaine exposure.
Disclosure: L. Smith: None. **M. Taniguchi:** None. **K. Dietz:** None. **M. Fontenot:** None. **B. Zirlin:** None. **K. Huber:** Part 1: I am on the scientific advisory board for Seaside Therapeutics. **C. Cowan:** None.

4. A Preclinical Examination of Behavioral and Neurotransmitter Specific Roles for the Ventral Tegmental Area in Reinforcer-Seeking and Drinking.

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Background: The ventral tegmental area (VTA) is a pivotal relay site within the brain's reinforcement circuit that has been shown to play a role in alcohol-motivated behaviors. The primary dopamine projection neurons within this system originate in the VTA and innervate several areas including the nucleus accumbens (NAc) and prefrontal cortex (PFC). In addition, the PFC has afferent glutamate projections to the VTA and the NAc. The following studies utilized two different operant behavior paradigms, one focusing on reinforcer-seeking and one on reinforcer self-administration, (both with an alcohol and a sucrose reinforcer solution) to elucidate regulation of these behaviors by the VTA, and the specific roles of dopamine and glutamate in this region.
Methods: The present experiments assessed the effects of microinjections of the glutamate (AMPA/kainate) antagonist CNQX and the dopamine D1-like antagonist SCH23390 into the posterior VTA (three doses of each drug and aCSF control). All experiments also included the transient chemical inactivation of this region using tetrodotoxin (TTX). In four separate experiments, (two Dopamine, two Glutamate, both with TTX) male Long Evans rats ($n = 6$-10/group) were trained to complete a single response requirement that resulted in access to a liquid reinforcer. Two of these experiments focused on the effects of VTA manipulations specifically on drinking and two specifically on seeking behavior. Separate groups of subjects were reinforced with either 10% alcohol or with 2% sucrose, and reinforcer access consisted of a single daily "binge" of twenty minutes of uninterrupted drinking following completion of the response requirement. To specifically assess seeking behavior, extinction "probe" sessions were used to determine the limit to responding in groups trained for either reinforcer solution.
Results: Prior to drug/TTX microinjections, alcohol-reinforced subjects were consuming ~0.45-0.65 g/kg ethanol and making ~50 responses during intermittent non-reinforced (extinction) sessions. Sucrose-reinforced groups had similar baseline response levels of responding. Overall in all four experiments, TTX inactivation of the VTA consistently and significantly ($p < .01$-$.05$) decreased reinforcer-seeking [for both alcohol (by 68-80%) and sucrose (by 76-79%)] but not drinking. Administration of the glutamate antagonist CNQX also significantly ($p < .01$) and dose-dependently decreased alcohol-seeking (by up to 53%), with no effect on sucrose-seeking and no effect on intake of either reinforcer. Administration of the dopamine antagonist SCH23390 had no effects on reinforcer-seeking and very moderately decreased intake of both alcohol and sucrose in a dose-dependent manner.
Discussion: Using this behavioral model, rats consume alcohol in "pharmacologically relevant" binges, and distinct assessment of seeking responses versus drinking behavior is possible. The chemical inactivation of the posterior VTA clearly implicated this region in reinforcer-seeking as opposed to self-administration. When assessing neurotransmitter-specific function of this region, there were significant effects of glutamate manipulation on seeking but not drinking behavior, and specifically on alcohol but not sucrose-seeking. Manipulation of dopamine function suggested a general effect on self-administration of both reinforcer solutions, but higher doses of the antagonist may be needed to reveal a significant effect and to confirm that it is specific to drinking but not seeking. Overall, the present findings provide support for the importance of posterior VTA glutamate activity specifically in alcohol-seeking behavior in animals consuming pharmacologically relevant amounts of alcohol. *This work was supported by funding

(R01AA016101) from the National Institute on Alcohol Abuse and Alcoholism.*
Disclosure: C. Czachowski: None.

5. Low Sensitivity or Level of Response to Ketamine Predicts High Alcohol Intake in Adolescent Rhesus Monkeys
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Background: Studies by Schuckit and other groups show that a low level of response to alcohol is present in a number of groups that are at increased risk for the development of alcoholism and that its potential to predict alcoholism risk is similar to the risk for alcohol abuse disorders found in children of alcoholics. Alcohol administration to measure level of response has has a number of inherent problems, including uneven absorbtion between individuals and there are also ethical concerns when administering it to children and humans at risk for alcohol disorders. In animal studies, it is often administered IV, but the range between BAC levels that produce intoxication and unconsciousness is narrow. Earlier observations from our laboratory suggest that the response to ketamine and other anesthetic agents such as isofluorane and pentobarbital are blunted in nonhuman primates that are at risk for high alcohol intake. Ketamine's neurobiological profile is similar to alcohol, with both acting on NMDA receptors. Ketamine also shows cross-tolerance with alcohol and unlike alcohol, it is relatively safe, even in doses that produce unconsciousness. This study was designed to assess to test level of response to ketamine (i.e., ketamine recovery time—time to sit up after dosing) as a predictor of future alcohol consumption. A second study looked at central serotonin functioning in infancy as a predictor of sensitivity to ketamine's anesthetic effects.
Methods: Subjects in the first study were 15 alcohol naïve adolescent male rhesus macaques. Each was exposed to 10 mg/kg of ketamine intramuscularly three times prior to their initial introduction to alcohol. Two weeks after the final ketamine exposure, they were allowed to consume an 8.4% palatable alcohol solution for two hours a day, five days a week for five weeks. In a second study with different subjects, CSF was removed from 16 infant rhesus monkeys (5 males and 11 females) when they were 60 days of age and assayed for monoamine metabolites. Three months later the alcohol naïve monkeys were administered 10mg/kg of ketamine intramuscularly and as in the first study, time to recovery was recorded. Each subject was tested twice, with two to three weeks between the trials.
Results: *Study 1*—After controlling for weight and ketamine dose, there was a significant correlation between the change in ketamine recovery time from the first and subsequent ketamine doses and future average weekly alcohol consumption. A large decrease in ketamine recovery time from the first dose to the subsequent doses of ketamine was highly predictive of alcohol intake across all five weeks ($r = 0.763$, 0.717, 0.710, $p < 0.05$, $n = 15$; $r = 0.929$, and $p < 0.889$, $p < 0.01$, $n = 11$). In both the first and the second study, the time to recover following the first dose was highly correlated with time to recover from the second (or third) dose. *Study 2*—In the second study, there was a strong, correlation between the CSF 5-HIAA concentrations taken when the subjects were 60 days of age and the average total time to recover from ketamine measured three months later ($r = 0.82$, $p < 0.001$, df 2/16). There was also a trend for MHPG to exhibit a correlation with time to wake up ($r = 0.65$, $p < 0.08$, df 2/16).
Discussion: A large decrease in the sensitivity to ketamine from an initial dose when compared to subsequent doses is predictive of high alcohol intake, possibly representing increased tolerance to ketamine's anesthetic effects following repeated exposure. This is similar recent studies from our laboratory showing that the change in the response to alcohol between the first and second exposure was a stronger predictor of future alcohol intake than simply looking at the level of response. In the second study we found that infant monkeys with low concentrations of CSF 5-HIAA appear to be less sensitive to the effects of ketamine, when compared to subjects with high CSF 5-HIAA concentrations. Our results suggest that animals with low CSF 5-HIAA concentrations appear to be less sensitive to the effects of a large variety of anesthetic agents that are cross tolerant with alcohol.
Disclosure: J. Higley: none. **A. Sorenson:** none. **B. Padro:** none. **J. Capitanio:** none. **L. Stephen:** none. **B. Christina:** none. **S. Suomi:** none.

6. Altered Levels of Nicotinic Acetylcholine and Metabotropic Glutamate Receptors Associated with Cue-Induced Nicotine-Seeking Behavior in a Rat Model of Drug Relapse
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Background: Nicotine addiction is a chronic relapsing disorder. Drug-associated environmental cues critically contribute to relapse to drug-seeking behavior in abstinent addicts. Tobacco smoking is particularly effective in establishing the conditioned incentive properties of associated environmental cues, as smoking rituals contain more drug-cue pairings (approximately 7000 per year in a moderate smoker) than other drugs of abuse. However, little is known about the neurobiological mechanisms underlying the motivational effects of nicotine cues. Based on previous animal research (Bespalov *et al.*, 2005 Dravolina *et al.*, 2007 Liu *et al.*, 2007)showing that pharmacological blockade of cholinergic neurotransmission via nicotinic acetylcholine receptors (nAChRs) and glutamatergic neurotransmission via metabotropic glutamate receptors (mGluRs) attenuates resumption of nicotine-seeking behavior, this study examined expression of certain subtypes of both nAChRs and mGluRs in specific key brain regions of rats after the cue-induced reinstatement test.
Methods: Male Sprague-Dawley rats were trained in 20 daily 1-h sessions to intravenously self-administer nicotine (0.03 mg/kg/infusion, free base) on an FR5 schedule. Each nicotine delivery was associated with a presentation of an auditory/visual stimulus so that the latter was established as a nicotine-conditioned cue. Then, in daily extinction sessions, lever responding was extinguished by withholding nicotine delivery and its cue. After extinction, the reinstatement tests were performed with response-contingent re-presentation of the cue without nicotine availability. Thirty min after the test session, brain samples were collected and processed for measuring expression of the nAChRs and the mGluRs.
Results: Re-exposure to nicotine cue effectively reinstated extinguished responses on the active, previously nicotine-reinforced lever, indicating reinstatement of nicotine-seeking behavior. Compared to the rats under extinction condition, the cue-reinstated animals showed altered levels of some subtypes of the nAChRs and the mGluRs in certain key brain regions. For example, a decreased mGluR5 was observed in the amygdala of the cue-exposed rats.
Discussion: This study demonstrates that a unique expression pattern of the nAChRs and the mGluRs was associated with cue-induced reinstatement of nicotine-seeking, indicating that the altered expression of these receptors may underlie the conditioned incentive properties of nicotine cues. These findings suggest that neurotransmission via the nAChRs and the mGluRs would be a promising target for development of pharmacotherapies to prevent smoking relapse associated with exposure to environmental cues. *Acknowledgement:* This study was supported by NIH grant R01DA017288 from the National Institute on Drug Abuse

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Disclosure: X. Liu: None. **B. Karolewicz:** None. **C. Jernigan:** None.

7. Gene × Early Environment Interactions determine Prefrontal Cortex DNA Methylation Status and Drug-Seeking in Adult Mice
Tod E. Kippin*, Kevin J. Dudley, Joannalee C. Campbell, Kyle L. Ploense, Xiang Li, Wei Wei, Dennis K. Gascoigne, John S. Mattick, Karen K. Szumlinski, Tim W. Bredy

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Background: Vulnerability to neuropsychiatric disease is widely believed to be influenced by gene-environment (GXE) interactions. Further, early life environment appears to have a profound impact on later life phenotypes including susceptibility to addiction. For example, exposure to stress during the prenatal period is associated with increased drug-seeking in animal models. Likewise, experiments carried out in mice and other rodent models have shown that genetic background can strongly influence later life phenotypic outcomes including differential vulnerability and resilience to prenatal stress (PNS). The present project employs a mouse model to examine the interaction between gene and early environment on epigenetic consequences in the medial prefrontal cortex (mPFC), a structure widely implicated in addiction and on drug seeking behavior in adulthood.
Methods: PNS was performed using repeated restraint stress on DBA/2J (D2) and C57/BL6J (B6) mice during the last week of pregnancy and offspring were studied in adulthood for DNA methylation in the mPFC or cocaine-induced conditioned place preference (CPP). For DNA methylation, genome-wide DNA methylation profiling was performed using a methyl-capture microarray approach to determine whether PNS leads to epigenetic changes in two genetically-distinct strains of mice. For CPP, mice were randomly selected to receive repeated pairings of vehicle (4 × 10 ml/kg saline, i.p.) and cocaine (4 × 3, 10, or 30 mg/kg, i.p.) with distinct context and then tested for their preferences for each environment.
Results: Widely varied differences in DNA methylation (2-fold differences or greater at 1621 promoters) were observed in non-PNS D2 and B6 adult male offspring, suggesting that underlying genetic background influences the epigenotype, which was further impacted by PNS. Interestingly, many of the genes displaying differential DNA methylation are well known to play a role in the neurodevelopmental process, and altered epigenetic regulation of these genes might therefore be responsible for the distinct behavioral phenotypes observed in these offspring in later life. Similar to the epigenetic effects, the impact of PNS on cocaine-seeking behavior also depended on genetic background (Treatment × Strain × Dose: $F_{2,\ 237} = 3.95$, $p < 0.05$). In B6 males, PNS elevated CPP induced by the 10 and 30 mg/kg doses of cocaine (ps < 0.05) but, in D2 males, PNS had no effect on CPP at any dose.
Discussion: The present data demonstrate the genetic background-dependent impact of PNS on both the pattern of methylation within promoter regions in the mPFC in a highly gene-specific fashion and level of drug-seeking behavior in adult mice. Although the relation between altered mPFC epigenome and enhanced drug-seeking behavior is unclear, the present findings indicate that there are PNS-resilient (D2) and PNS-susceptible (B6) genotypes. Further, recombinant inbred strains based on genotypes of differing PNS susceptibility may be useful for examining the nature of GXE interactions and their molecular underpinnings that determine adult addiction vulnerability.
Disclosure: T. Kippin: None. **K. Dudley:** None. **J. Campbell:** None. **K. Ploense:** None. **X. Li:** None. **W. Wei:** None. **D. Gascoigne:** None. **J. Mattick:** None. **K. Szumlinski:** None. **T. Bredy:** None.

8. Novelty Seeking Mediates Early Life Stress Effects on Psychostimulant Place Preference Conditioning in Monkeys
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Background: Novelty seeking behavior has been linked to psychostimulant drug use and addiction. Novelty seeking in monkeys is increased by exposure to early life stress. Here we examine whether novelty seeking mediates early life stress effects on the acquisition and subsequent extinction of psychostimulant place preference conditioning.
Methods: Female squirrel monkeys were randomized to early life stress (N = 7) or no-stress (N = 8) conditions conducted from 17 to 27 weeks of age. Previously published measures of novelty seeking behavior collected at 9 months and 2.5 years of age were analyzed as predictors of cocaine place preference conditioning and extinction in adulthood at 4.4 years of age. Impulsivity and anxiety-like behavior were also included in the analysis because these factors have been shown to predict psychostimulant drug use in humans and animal models.
Results: Monkeys from both of the early life treatment conditions acquired an equally robust cocaine-induced place preference. Significant differences were subsequently discerned during extinction trials ($P < 0.05$). Monkeys exposed to early life stress maintained their cocaine-induced place preference several months longer than monkeys from the early life no-stress condition. Prior measures of novelty seeking ($\eta = 1.19$, $P = 0.007$) but not impulsivity ($\beta = 0.08$, $P = 0.737$) nor anxiety-like behavior ($\eta = 0.56$, $P = 0.114$) predicted resistance to extinction.
Discussion: These results indicate that early experience-dependent novelty seeking mediates the effects of stress on resistance to extinction of a conditioned place preference for cocaine. In this regard, novelty seeking is distinct from impulsivity and anxiety-related biomarkers of psychostimulant drug seeking behavior.
Disclosure: D. Lyons: None. **C. Buckmaster:** None. **A. Schatzberg:** Part 1: Consultant to: BrainCells CeNeRx CNS Response Eli Lilly GSK Jazz Lundbeck McKinsey Neuronetics NovaDel Pharma-NeuroBoost Sanofi-Aventis Takeda Equity in: Amnestix Corcept (co-founder) Forest Merck Neurocrine Pfizer Somaxon Speaker for: Pfizer Intellectual Property: Named inventor on pharmacogenetic use patents on prediction of antidepressant response and glucocorticoid antagonists in psychiatry, Part 2: Amnestix, Corcept, Forest, Merck, Neurocrine, Pfizer, and PharmaNeuroBoost Intellectual Property Named inventor on pharmacogenetic use patents on prediction of antidepressant response and glucocorticoid antagonists in psychiatry., Part 3: PharmaNeuroBoost.

9. Roles for Beta-2 Adrenergic Receptor Activation in the BNST and CRF-R1 Receptor Activation in the VTA in Stress-Induced Reinstatement of Cocaine Seeking Following Long-Access Self-Administration in Rats
John R. Mantsch*, Jordan M. Blacktop, Yazmin Figueroa-Guzman, Oliver Vranjkovic

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Background: Understanding the neurobiological pathways that contribute to relapse in recovering cocaine addicts is crucial to the development of new and more effective pharmacotherapeutic approaches for the management of addiction. Previous work has suggested roles for corticotropin releasing factor (CRF) and beta-adrenergic receptors in stress- but not cocaine-induced reinstatement in rodent models of relapse. Further, a circuit that includes a putative projection from the ventrolateral bed nucleus of the stria terminalis (vlBNST) to the ventral tegmental area (VTA) has been proposed to mediate stress-induced relapse. Recently, we have

reported that the ability of stress to trigger cocaine seeking is established by excessive drug use (as modeled using the long-access/LgA self-administration approach) and requires CRF-R1 receptor activation in the VTA. Here we examine the relationship between beta adrenergic receptor (AR) subtypes in the vlBNST and CRF receptor subtypes in the VTA and their contributions to stress-induced reinstatement in LgA rats.

Methods: Adult male Sprague-Dawley rats were trained to self-administer cocaine (1.0 mg/kg/inf) by pressing a lever under a FR4 schedule and, after acquisition, were provided daily access under LgA conditions (6 hrs/day; mean daily intake = approximately 70 mg/kg/day) for 14 days prior to undergoing daily 2-h extinction sessions. Once the extinction criteria were met (<15 responses/2-h session for 2 consecutive days), rats were tested for reinstatement following stress (footshock: 0.5 mA, 0.5 s duration average every 40 s over a 15-min period) or in response to bilateral delivery of drugs directly into the VTA (12° angle, -5.6 mm a/p from bregma, ± 2.2 mm m/l, -6.7 mm d/v) or vlBNST (15° angle, -0.6 mm a/p, ± 3.5 mm m/l, -5.2 mm d/v) via microinjection (0.25 or 0.5 μl/side over 1 min). In some cases reinstatement testing was preceded by local delivery of antagonists into the vlBNST or the VTA.

Results: Reinstatement by footshock was blocked by administration of the CRF-R1 receptor antagonist, antalarmin (500 ng/side; $n = 6$), but not the CRF-R2 antagonist, astressin-2B (500 ng/side; $n = 8$), into the VTA. Two-way repeated measures ANOVA showed a significant interaction between footshock and intra-VTA antalarmin pretreatment ($F_{1,5} = 6.294$; $P = 0.05$) but not between footshock and astressin-2B. Footshock produced significant reinstatement following pretreatment with vehicle or astressin-2B ($P < 0.05$ vs. extinction) but not antalarmin, and antalarmin, but not astressin-2B, significantly decreased shock-induced reinstatement ($P < 0.01$). Bilateral intra-VTA delivery of the CRF-R1 receptor agonist, cortagine (100 ng/side; $n = 5$), but not the CRF-R2 receptor agonist, rat urocortin II (250 ng/side; $n = 5$) reinstated cocaine seeking ($P < 0.05$ vs. vehicle and extinction). Footshock-induced reinstatement was also blocked by administration of the beta-2 AR antagonist ICI 118,551 (1 nM/side; $n = 5$), but not the beta-1 AR antagonist betaxolol (1 nM/side; $n = 5$), delivered into the vlBNST. Two-way repeated measures ANOVA showed a significant interaction between footshock and intra-BNST ICI 118,551 pretreatment ($F_{1,4} = 15.026$; $P < 0.05$) but not between footshock and intra-BNST delivery of betaxolol. Footshock produced significant reinstatement following pretreatment with vehicle or betaxolol ($P < 0.05$ vs. extinction), but not ICI 118,551, and ICI 118,551, but not betaxolol, significantly decreased shock-induced reinstatement ($P < 0.01$). Further, bilateral delivery of the beta-2 AR agonist, clenbuterol (10 ng/side; $n = 4$) into the vlBNST reinstated cocaine seeking ($P < 0.05$ vs. vehicle and extinction).

Discussion: These findings are consistent with reports that a putative projection from the vlBNST to the VTA is critical for drug seeking triggered by stressful stimuli and suggest that beta-2 ARs activated upon stress-induced release of norepinephrine into the vlBNST and CRF-R1 receptors activated upon CRF release into the VTA contribute to stress-induced relapse in cocaine addicts. The results of experiments examining the ability of antagonism of CRF-R1 receptors in the VTA to prevent reinstatement by clenbuterol delivered into the vlBNST will be reported. Lastly, our findings suggest that the CRF-R1 receptor and the beta-2 AR may serve as viable medicinal targets for the management of relapse, particularly in individuals whose drug use is stress driven.

Disclosure: J. Mantsch: Part 1: Co-founder and consultant, Promentis Pharmaceuticals, Milwaukee, WI Consultant, WIL Research, Inc., Ashland, OH, Part 2: Promentis Pharmaceuticals, Part 3: Promentis Pharmaceuticals, Part 4: Promentis Pharmaceuticals. **J. Blacktop:** None. **Y. Figueroa-Guzman:** None. **O. Vranjkovic:** None.

10. Relapse to Cocaine-seeking Normalizes Elevated Phospho-CREB and Phospho-Ser845-GluR1 in the Prefrontal Cortex and Phospho-Ser9-Synapsin in Nucleus Accumbens after One Week of Abstinence

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Background: Previously, we demonstrated that phospho-ERK (p-ERK) and phospho (p)-CREB were decreased in the dorsomedial prefrontal cortex (dmPFC) 2 hr after the end of cocaine self administration (SA) and that intra-dmPFC infusion of BDNF reversed this ERK/CREB shutoff and suppressed cocaine-seeking (Whitfield *et al*., 2011). However, after 7 days of abstinence from cocaine, p-CREB, but not p-ERK, in the PFC was significantly elevated. Because CREB is phosphorylated by multiple kinases including PKA, the responses of multiple PKA-CREB targets in the dmPFC and nucleus accumbens (NAc) to abstinence and relapse to cocaine-seeking were investigated in this study.

Methods: Chronic intravenous catheters were implanted into the right jugular vein of anesthetized rats. After 5 days of recovery, rats were assigned to cocaine or yoked-saline control groups and placed in standard chambers to self-administer cocaine daily during 2 hr sessions for 10 days on an FR1 schedule of reinforcement. Yoked rats received a saline infusion whenever the matched subject received a drug infusion. After 7 days of abstinence, rats were decapitated with or without a 30 min context cocaine-seeking test in the drug-paired chamber. Brains were extracted and the dmPFC and NAc were dissected and processed for Western blotting.

Results: Cocaine abstinence caused an increase in p-CREB and the PKA target, p-Ser845-GluR1, but not in p-ERK, immunoreactivity in the dmPFC and also increased presynaptic PKA/CaMKI-mediated pSer9-synapsin levels in the NAc. In contrast, relapse to cocaine-seeking after abstinence normalized the levels of all these phosphoproteins.

Discussion: These results suggest that cocaine-seeking regulates p-CREB, pSer9-GluR1, and pSer9-synapsin in the PFC-NAc pathway in a PKA-dependent manner. Identification of the potential molecular mechanisms underlying these phosphorylation effects will elucidate their role in relapse behaviors and aberrant neurotransmission after cocaine self-administration.

Disclosure: W. Sun: None. **J. McGinty:** None.

11. Female Vulnerability to Episodic and Continuous Social Stress: Dopamine and Cocaine Self-Administration

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Background: Women are more vulnerable to stressors inducing certain types of psychiatric disorders, including major depression. Chronic stress disorders are often associated with substance use disorders, and the mechanisms by which stressful experiences alter drug taking have yet to be studied. Evidence suggests that certain types of social stress can enhance neuronal adaptation in drug reward pathway, particularly in the nucleus accumbens (NAc). Recently we have demonstrated that in males, chronic social defeat stress caused a blunted response to a cocaine challenge in extracellular dopamine (DA) in the NAc, whereas intermittent social defeat stress enhanced DA response to the cocaine challenge. Rats that experienced chronic social defeat stress self-administered fewer infusions of cocaine during a 24-h "binge" of cocaine self-administration as opposed to rats that took more cocaine after they experienced intermittent social defeat stress. These data prompted us to examine the effects of social defeat stress on 1) DA response to cocaine, 2) behavioral sensitization, and 3) cocaine self-administration in female rats.

Methods: Female Long-Evans rats were examined for baseline measurements of weight, preference for saccharin, and estrous cycles. Once the baseline measurements were stable, the rats were assigned to either intermittent episodic, continuous social stress or non-stressed control groups. For chronic social subordination stress, each experimental female rat confronted a nursing dam for 30 min twice daily for 21 days. In the intervals between the confrontations, the rat was housed in a wire-mesh protective cage inside the opponent's home cage to maximize the effects of being threatened by the resident aggressor. For intermittent social defeat stress, each rat experienced four episodes of direct confrontation separated by 72 hours. Ten days after the last confrontation, the experimental rat was challenged with 10 mg/kg of cocaine to examine either behavioral sensitization or the extracellular DA and serotonin (5-HT) responses in the NAc, using HPLC. Thereafter, the rats acquired cocaine (0.75 mg/kg/infusion) self-administration and were maintained on a FR 5 schedule, followed by a progressive ratio schedule (0.3 mg/kg/infusion). Finally, a 24-h 'binge' test was performed, reinforcing each fifth response with a 0.2 mg/kg/infusion of cocaine. For some rats, the 'binge' test consisted of a variable dose (0.375, 0.75 and 1.5 mg/kg/infusion) sequence.
Results: 1) DA response to cocaine: Cocaine increased extracellular DA in the NAc, and this increment was significantly reduced in rats which experienced continuous social subordination stress. The extracellular 5-HT response to cocaine was also blunted in continuously stressed rats. These rats exhibited less preference for saccharin, lower weight gain, and disruption of estrous cycles. In contrast, these behavioral parameters were not influenced in rats which experienced intermittent episodes of social defeat stress. The effect of cocaine challenge on extracellular DA was long-lasting in these rats. 2) Locomotor activity as assessed by duration of walking behavior was significantly increased in rats which experienced intermittent social defeat stress. Particularly, rats in the estrous phase showed a prominent increase in duration of walking behavior. The increased locomotor activity persisted for 30 min after the acute cocaine injection. 3) Rats which experienced intermittent episodes of social defeat stress had higher number of cocaine infusions when they were in proestrus. The 24-h 'binge' test did not reveal stress effects in females that had experienced intermittent episodes of social defeat.
Discussion: Two types of social stress engendered distinct patterns of neurochemical and behavioral profiles in females that differed also from those in males. Particularly, the blunted DA and 5-HT responses to cocaine and the anhedonia-like behavioral deficits during and after continuous social stress suggest that these female rats exhibit a depressive-like phenotype. Disruption of endocrine cyclicity, suppressed preference for sweets and shorter cocaine binges are consistent with cardinal symptoms of a depressive-like phenotype. In contrast, episodic social defeat stress which resulted in behavioral sensitization did not intensify cocaine self-administration, suggesting that – in contrast to males – females may show a clear dissociation between stress-induced behavioral sensitization and intense cocaine binges.
Disclosure: A. Shimamoto: None. **E. Holly:** None. **J. DeBold:** Part 1: none. **K. Miczek:** none.

12. Brain-Regional Neuroadaptations in SK Channels following Chronic Alcohol Exposure and Withdrawal

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Background: Dependence upon alcohol is associated with high rates of alcohol consumption and brain hyperexcitability following cessation of drinking. While recent evidence implicates significant roles for key brain structures within cortical-limbic-basal ganglia circuitry in mediating escalation of drinking and withdrawal hyperexcitability associated with dependence, the neural mechanisms that underlie such neuroadaptations in ethanol-dependent mice are unknown. Characterizing neuroadaptations in these brains regions may lead to identification of novel therapeutic targets to reduce the serious consequences of dependence, including withdrawal symptoms and relapse to excessive drinking in dependent individuals. A recently identified potential target for treating alcoholism is the small-conductance calcium-activated potassium (SK) channel. SK channels functionally couple to synaptic NMDA receptors in pyramidal neurons and modulate intrinsic excitability of GABAergic neurons. This study explored how neuroadaptive changes in SK channels in key brain regions contribute to escalation of ethanol drinking and withdrawal seizure activity in ethanol-dependent mice.
Methods: Adult male C3H/Hecr and C57BL/6J mice were exposed to chronic ethanol vapor in inhalation chambers delivered in either a continuous (64 hr) or intermittent (4 weekly cycles of 16 hr/day/4 days a week) treatment regimen. Following chronic ethanol exposure, postsynaptic density-enriched fractions were prepared from tissue punches taken from brain regions within the addiction neurocircuitry (medial prefrontal corex (mPFC), nucleus accumbens (NAc) core, rostroventral striatum (rvSTr), hippocampus, amygdala, and substantia nigra pars reticulata (SNr)). Neuroadaptive changes in NMDA and GABA receptor and SK channel expression levels were assessed by Western blot analysis. Whole-cell patch clamp electrophysiology was used to determine if continuous ethanol exposure disrupts the SK channel-NMDA receptor feedback loop. We also determined the ability of SK channel blockers (apamin) and positive modulators (chlorzoxazone (CZX), 1-EBIO) to affect drinking and withdrawal hyperexcitability and neurotoxicity using in-vivo and in-vitro models.
Results: Continuous chronic ethanol exposure significantly reduced synaptic expression of SK2 potassium channels and increased GluN1 and GluN2B subunits of the NMDA receptor in hippocampus of C3H/Hecr mice. This was associated with an uncoupling of the calcium-dependent SK channel-NMDA receptor negative feedback loop in dendritic spines. Chronic intermittent ethanol (CIE) exposure in C57BL/6J mice down-regulated expression of synaptic SK channels in hippocampus, as well as SK channels in the NAc core and mPFC. Preliminary evidence also suggests that CIE exposure decreased expression of SK channels and the á4 subunit of the GABA-A receptor in the rvSTr. Changes in SK channels or NMDA receptor subunits were not observed in the amygdala or SNr following CIE exposure. Positive modulation of SK channels prevented in-vitro withdrawal hyperexcitability and neurotoxicity, and systemic administration of 1-EBIO attenuated the severity of handling-induced convulsions during acute ethanol withdrawal. Finally, microinjection of apamin into the NAc core markedly increased voluntary ethanol consumption in non-dependent, but not in ethanol-dependent mice in a limited-access two-bottle choice paradigm.
Discussion: In two mouse models of ethanol dependence, we observed neuroadaptations in proteins that regulate intrinsic excitability and synaptic plasticity in key brain regions within the addiction-relevant cortical-limbic-basal ganglia circuitry. SK channel expression was significantly down-regulated by CIE exposure in hippocampus, mPFC, NAc core, and rvSTr. Restoring SK channel activity prevented in-vitro withdrawal excitability and neurotoxicity in hippocampus and reduced the severity of handling-induced convulsions in ethanol-dependent mice. Blocking NAc core SK channels increased voluntary drinking only in non-dependent mice, suggesting that CIE-induced reductions in SK channels in the NAc core contribute to excessive drinking in dependent mice. Taken together, these data suggest that SK channels represent a promising, novel target for drug development, with potential for impacting treatment of alcohol dependence.

Disclosure: P. Mulholland: None. **W. Griffin III:** None. **H. Becker:** None.

13. Double-Dissociation in the Control over the Acquisition and Performance of Cocaine Seeking by the Dorsomedial and Dorsolateral Striatum

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Background: The transition from goal-directed to habitual drug seeking behavior has been hypothesized to depend upon different domains of ventral and dorsal striatal circuitry. The present study investigated the involvement of dopamine-dependent mechanisms in the posterior dorsomedial striatum (pDMS) and anterior dorsolateral striatum (aDLS) in the acquisition of and later, well-established performance of cocaine-seeking behavior.
Methods: Rats were trained to self-administer cocaine (0.25 mg/infusion) under a continuous reinforcement (FR1) schedule with infusions occurring in the presence of a 20-s light conditioned stimulus (CS). Following stabilization of this drug-taking response, dopamine transmission was blocked in either the aDLS or pDMS via bilateral intracranial infusions of the D1/D2 dopamine receptor antagonist α-flupenthixol (0, 5, 10, or 15 μg/side) during 15-min cocaine seeking test sessions in which each response was reinforced by a 1-s cocaine-associated CS presentation alone. For half the rats, the response requirement for cocaine was then gradually increased over successive training sessions from FR1 to a second-order schedule [FI15(FR10:S)] in which cocaine seeking was maintained over 15-min delays by 1-s response-contingent presentations of the cocaine-associated light on every 10^{th} lever press. The remaining rats were maintained on a FR1 schedule with the number of cocaine infusions limited to match precisely the cocaine exposure of those rats for which the response requirement was increased under second-order schedule conditions. Following 15 fifteen training sessions under these conditions, seeking tests with dopamine receptor blockade in the aDLS or pDMS were again conducted. For the rats whose behavior was shifted to the second-order schedule, every 10^{th} lever press resulted in the 1-sec light CS presentation alone during the tests. For the rats that were maintained on the FR1 schedule, each lever press resulted in the 1-sec light CS presentation alone during the tests.
Results: Early acquisition of cocaine seeking was dose-dependently reduced by α-flupenthixol infusions into the pDMS, but not by infusions into the aDLS. The performance of late-stage, or well-established, cocaine seeking following an increase in the response requirement to the second-order schedule were, in contrast, reduced by α-flupenthixol infusions into the aDLS, but not the pDMS. Notably, rats that continued to respond under FR1 schedule conditions but which had received the same amount of cocaine over their drug-taking history continued to show the pattern of results found at the early stage of cocaine seeking, with a reduction of responding when α-flupenthixol was infused into the pDMS but not in the aDLS.
Discussion: Combined, these results show that dopamine transmission in the pDMS is required for the acquisition of presumably goal-directed cocaine seeking, whereas aDLS dopamine-dependent mechanisms become dominant to control cocaine seeking when it is well-established and under the control of cocaine associated stimuli. These findings indicate a regional posterior-to-anterior and medial-to-lateral shift in dorsal striatal control over behavior and, we hypothesize, reflect the transition from goal-directed to habitual stages of cocaine seeking. However, if rats continue to seek and take cocaine under continuous reinforcement conditions in which each response, rather than every 10 responses is followed by CS presentation, the seeking behavior remains dependent upon dopamine signaling in the pDMS rather than the aDLS. This double dissociation in the control over cocaine seeking by the pDMS and aDLS suggests the importance of dynamically regulated dorsal striatal circuitries that are differentially engaged under the goal-directed or stimulus-response nature of cocaine seeking.
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Disclosure: J. Murray: None. **D. Belin:** None. **B. Everitt:** None.

14. A Mutation in the Circadian Gene *CLOCK* Increases the Vulnerability for Cocaine Addiction in Mice.

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Background: Disruption of sleep and circadian rhythms is a symptom common to many psychiatric disorders, including drug dependence. McClung *et al*., (2005) identified a key role for the *CLOCK* gene in drugs of abuse. Mice bearing a dominant negative mutation in the *CLOCK* gene (CLOCKΔ19 mice) display increased locomotor responses to novelty, robust cocaine sensitization, and express conditioned place preference for cocaine to a greater extent at lower doses. Furthermore, these mice exhibit reduced anxiety- and depression-like behavior, increased intracranial self-stimulation at a lower threshold, and increased dopaminergic cell activity in the ventral tegmental area (VTA)(McClung *et al*., 2005; Roybal *et al*., 2007). Since many of these behavioral phenotypes are correlated with increased vulnerability to cocaine's rewarding effects, we sought to determine if this hyperhedonic phenotype extends to cocaine self-administration. This clinically relevant paradigm is a direct measure of individual sensitivity to the reinforcing and motivational properties of cocaine. Upon completion of cocaine self-administration schedules, we evaluated extinction and cue-induced reinstatement of cocaine-seeking responses to determine if CLOCK is important in a model of relapse-like behavior.
Methods: Two separate serial testing procedures were carried out with male CLOCKΔ19 mice and their wild-type (WT) littermates ($n = 7$-10/genotype/schedule) with experiments performed at either Zeitgeber time 2 (ZT; lights on at ZT0), followed by ZT14 or ZT14 only. Testing began with acquisition of sucrose pellet self-administration, surgical implantation of an indwelling intravenous catheter, acquisition of cocaine self-administration (FR1, 0.5mg/kg/infusion), and either self-administration dose response testing on a fixed ratio (FR; descending dose presentation of 1.0, 0.5, 0.125, 0.063, and 0 mg/kg/infusion) or on a progressive ratio (PR; counterbalanced presentation of 1.0, 0.5, and 0.25 mg/kg/infusion) schedule of cocaine reinforcement. Drug-seeking and cue-induced reinstatement of responding was probed either 7 or 28 days after the end of the FR or PR schedule testing procedure, respectively.
Results: We found that both WT and ClockΔ19 mice showed similar acquisition of sucrose pellet self-administration at ZT2 and ZT14. However, ClockΔ19 mice exhibit a greater propensity to initiate cocaine use (regardless of time of day sessions occurred), reduced latency to acquire cocaine self-administration, and elevated cocaine intake during acquisition (main effect of genotype: % reaching acquisition - $F(1,56) = 41.25$, $p < 0.001$; acquisition latency - $F(1,13) = 16.83$, $p < 0.01$; cocaine intake - $F(1,54) = 21.98$, $p < 0.0001$). In the FR1 dose-response schedule, ClockΔ19 and WT mice earned more cocaine infusions at the lower end of the dose scale (main effect of dose – $F(1,72) = 19.1$, $p < 0.0001$). There was an upward shift in the dose-repsonse curve, where ClockΔ19 mice earned more infusions overall than did WT mice (main effect of genotype – $F(5,72) = 2.62$, $p < 0.05$). In the PR

dose-response schedule, ClockΔ19 mice had a higher breakpoint, earning significantly more infusions per session than WT mice ($F(1,48) = 9.16$, $p < 0.01$). ClockΔ19 mice had greater context- and cue-induced cocaine seeking behavior during protracted withdrawal (context - $F(1,84) = 8.75$, $p < 0.001$; cue - $F(1,14) = 6.68$, $p < 0.05$). Together, these results suggest cocaine is a more efficacious reinforcer in ClockΔ19 mice than in WT mice.
Discussion: This study demonstrates that a single mutation in the CLOCK gene is sufficient to produce vulnerability for cocaine addiction in mice. There is strong evidence from the literature suggesting that these CLOCK-mediated effects on cocaine reward are likely related to augmented dopaminergic activity. Increased excitability of VTA DA neurons is linked with increased levels of reward. DA cell firing rates are increased in ClockΔ19 mice, a phenomenon which also occurs in mice that have reduced CLOCK expression in the VTA due to RNA interference. Moreover, genes involved in DA synthesis, metabolism, and release, which are known to influence dopaminergic activity in the VTA, are up-regulated in ClockΔ19 mutants and in mice that have reduced CLOCK expression in the VTA due to RNA interference (McClung *et al.*, 2005; Mukherjee *et al.*, 2010). Spencer *et al.* (submitted) recently reported direct evidence that CLOCK acts as a negative regulator of TH transcription. Therefore, it is conceivable these alterations underlie the increased reinforcing efficacy of cocaine seen in ClockΔ19 mice.
Disclosure: A. Ozburn: None. **E. Larson:** None. **D. Self:** Part 1: Shareholder, Noorik Biopharmaceuticals Ltd.; US/International patents, Janus Pharmaceuticals Inc. **C. McClung:** Part 1: Honoraria from Servier; Consulting fees and research contract with Pfizer; Honoraria and research contract with GlaxoSmithKline.

15. Protracted Withdrawal from Cocaine Self-Administration Flips the Switch on 5-HT_{1B} Receptor Modulation of Cocaine Reinforcement

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Background: The role of serotonin-1B receptors (5-HT_{1B}Rs) in modulating cocaine abuse-related behaviors has been controversial due to discrepancies between pharmacological and gene knockout approaches, and opposite influences on cocaine self-administration versus cocaine-seeking behavior. We hypothesized that 5-HT_{1B}R-modulation of these behaviors may vary depending on the stage of the addiction cycle.
Methods: To test this hypothesis, we examined the effects of increased 5-HT_{1B}R tone via receptor over-expression or agonist treatment either during maintenance of cocaine self-administration (i.e. daily intoxication stage) or following 21 days of forced abstinence (i.e. protracted withdrawal stage). Rats trained to self-administer cocaine over 17, 2-h daily sessions received microinfusions of a viral vector expressing either green fluorescent protein (GFP) or GFP and 5-HT_{1B}R into the medial nucleus accumbens shell and 4 days later they were tested for cocaine intake on both fixed (FR) and progressive (PR) ratio schedules of reinforcement.
Results: 5-HT_{1B}R-gene transfer shifted the dose–response curve for cocaine self-administration upward and to the left and increased break points and cocaine intake on a PR schedule in rats tested during maintenance, suggesting increased reinforcing effects of cocaine. In contrast, following 21days of forced abstinence 5-HT_{1B}R-gene transfer attenuated break points and cocaine intake on a PR schedule of reinforcement, as well as cue- and cocaine-primed reinstatement of cocaine-seeking behavior. This pattern of changes suggests a decrease in cocaine reinforcement and attenuation of the incentive motivational effects of cocaine-priming injections and cocaine-associated cues during protracted withdrawal. We found converging evidence for the latter in a subsequent pharmacological experiment in which acute systemic administration of the 5-HT_{1B}R agonist CP 94,253 (5.6 mg/kg, i.p.) during protracted withdrawal attenuated break points and cocaine intake on a PR schedule.
Discussion: Collectively these results suggest that 5-HT_{1B}Rs undergo a change in modulatory influence over cocaine-taking behavior, with a facilitative influence during periods of active drug use (i.e. maintenance/intoxication) in striking contrast to an inhibitory influence during protracted withdrawal. These findings suggest that targeting 5-HT_{1B}Rs may lead to a novel treatment for cocaine dependence and that the therapeutic efficacy of these treatments may vary depending on the stage of the addiction cycle.
Disclosure: N. Pentkowski: None. **T. Cheung:** None. **W. Toy:** None. **M. Adams:** None. **J. Neumaier:** None. **J. Neisewander:** None.

16. Alpha-2 Adrenergic Agonists and CRF Antagonist Block the Negative Emotional Signs of Naloxone-Precipitated Morphine Withdrawal in Rats

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Background: Drug abstinence in humans can elicit emotional or affective symptoms such as depressed mood/dysphoria and anxiety, which contribute to the maintenance of use and relapse. To determine the role of dysregulated stress systems (corticotropin-releasing factor [CRF] and norepinephrine [NE]) in mediating negative emotional withdrawal signs in rats, we examined the ability of alpha-2 adrenergic receptor agonists (clonidine, UK14304) and a CRF antagonist (MPZP) to attenuate dysphoria-like (elevation in brain stimulation reward [BSR] thresholds) and anxiety-like (decreased exploration in the elevated plus maze [EPM]) signs of withdrawal from acute and chronic morphine dependence.
Methods: The EPM and BSR procedures are previously described (Zhang & Schulteis 2008, Pharmacol Biochem Behav 89:392; Liu & Schulteis 2004, Pharmacol Biochem Behav 79:101). Wistar rats were handled and injected with either vehicle or morphine for 4 consecutive days. Three injection regimens based on the cited prior studies were employed: Morphine Naive (4 vehicle injections), Single Morphine (3 vehicle injections, 4th injecti on 5.6 mg/kg [BSR]) or 10 mg/kg [EPM]), or Repeat Morphine (all 4 injections with same doses of morphine). Naloxone (0.33-1 mg/kg) was administered 4 hr [BSR] or 8 hr [EPM] after the 1st (Acute) or 4th (Repeat) injection of morphine. Clonidine (5-20 μg/kg), UK 14304 (10-100 μg/kg), MPZP or their vehicle controls were injected 60 min prior to naloxone. Due to limited supply of MPZP its effects were only examined under Repeat conditions.
Results: Naloxone-precipitated morphine withdrawal: (1) increased anxiety-like behavior in the EPM as evidenced by decreased time spent on the open arms and (2) increased dyspohria-like behavior as evidenced by elevated BSR thresholds, with greater magnitude of both signs following Repeat vs. Single Morphine. Clonidine (20 μg/kg) partially attenuated the decreased open arm time produced by precipitated withdrawal from Acute morphine, but as expected also produced significant motoric effects (decreased closed arm entries, $p < 0.05$ vs. Morphine Naïve). The more selective alpha-2 adrenergic agonist UK14304 also dose-dependently attenuated anxiety-like behavior in rats undergoing naloxone-precipitated withdrawal from Acute Morphine ($F(3,37) = 3.84$, $p = 0.017$), with the high dose of UK14304 50 μg/kg showing a full blockade. Lower doses of UK14304 (10 and 20 μg/kg) reversed anxiety-like behavior in Repeat Morphine rats ($F(3,42) = 3,54$, $p = 0.022$). A similar

pattern of effects with alpha-2 adrenergic agonists was observed for elevated BSR thresholds during withdrawal. After Acute Morphine, clonidine (20 μg/kg) restored BSR thresholds to 105% of baseline (from 125% in rats undergoing precipitated withdrawal but given vehicle in place of clonidine). UK14304 dose-dependently reversed elevated BSR thresholds for withdrawal from Repeat morphine ($F(4,44) = 4.61$, $p < 0.01$) and partially attenuated elevated BSR thresholds following Acute morphine. Similarly, the CRF-R1 antagonist MPZP attenuated the anxiogenic effects of precipitated withdrawal from Repeat Morphine at the highest dose tested (20 mg/kg; $p < 0.05$ vs.precipated withdrawal group receiving vehicle in place of MPZP). MPZP 10 mg/kg significantly blocked naloxone-precipitated BSR threshold elevations, with a 35% reduction from peak withdrawal (143% of baseline) in the withdrawing controls given vehicle not MPZP [$F(1,36) = 7.97$, $P < 0.008$].

Discussion: Reducing NE tone or reducing CRF-R1 receptor signaling blocks both the dysphoric and anxiogenic effects of naloxone-precipitated morphine withdrawal. Interestingly, lower doses of UK14304 were effective in reversing withdrawal effects in both BSR and EPM paradigms under conditions of chronic dependence, suggesting that the NE system mediates the negative emotional withdrawal signs to a greater degree under chronic vs. acute morphine regimens. Taken together these studies indicate that both NE and CRF stress systems mediate in part the negative emotional state produced by morphine withdrawal.

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17. Electrical Stimulation of the Granular Insular Cortex attenuates Nicotine-Taking and -Seeking Behavior

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Background: Nicotine is the primary psychoactive component of tobacco smoke resulting in tobacco addiction and is also reliably self-administered by numerous animals including rats. The insular cortex has been of some interest recently in the field of addiction and we have previously demonstrated that pharmacological inactivation of the granular insular (GI) cortex is able to block nicotine taking and seeking behaviors in a rat model of nicotine-self administration. In the present study, we explored the potential of modulating activity in the region of the GI using electrical stimulation in order to affect nicotine self-administration and reinstatement behavior.

Methods: After being implanted with jugular vein catheters, Long-Evans rats were trained to self-administer nicotine (0.03 mg/kg/infusion) under a fixed ratio-5 (FR-5) schedule of reinforcement followed by a progressive ratio (PR) schedule. Following the implantation of electrodes into the GI, evaluation of the effect of GI stimulation (200 mA, 130 Hz, 90 μs pulse width) was performed on nicotine self-administration under both FR-5 and PR schedules. Animals were then extinguished in multiple sessions by the removal of nicotine and nicotine-associated light cues. Once lever-pressing behavior was extinguished, GI stimulation was examined for its effect on reinstatement of nicotine-seeking behavior induced by the presentation of nicotine-associated light cues or priming injections of nicotine (0.15 mg/kg, subcutaneous). For all testing, GI stimulation was conducted throughout the self-administration or reinstatement sessions and was compared statistically to similar sessions where the animals were hooked up to the stimulator but did not receive stimulation.

Results: Electrical stimulation of the GI significantly attenuated nicotine taking, under both FR-5 ($F_{(3,11)} = 12.35$, $p < .0001$ repeated measures (RM) ANOVA; $p < .05$ Bonferroni comparison to sham condition) and PR ($F_{(3,10)} = 14.79$, $p < .0001$ RM ANOVA; $p < .01$ Bonferroni comparison) schedules of reinforcement. Following extinction, electrical stimulation of the GI was found to also significantly attenuate nicotine seeking behavior induced by both nicotine-associated light cues ($F_{(2,9)} = 21.54$, $p < .001$ RM ANOVA; $p < .05$ Bonferroni comparison) and nicotine priming injections ($F_{(2,9)} = 17.03$, $p < .001$ RM ANOVA; $p < .05$ Bonferroni comparison). These effects appear to be specific to nicotine-associated behaviors, as GI stimulation did not appear have any effect on food self-administration ($F_{(2,5)} = 0.2763$, $p > .05$).

Discussion: Electrical stimulation of the GI was capable of decreasing nicotine-taking under both schedules of reinforcement as well as nicotine seeking under both methods of inducing reinstatement. It must be noted that these effects appear to be weaker than those observed previously with pharmacological inhibition of the GI; however, we cannot be sure as to whether this difference is due to the parameters of the stimulation or the general mechanism of electrical stimulation itself. Regardless, our results suggest that modulating the activity of the insular region for nicotine dependence should be further explored in order to determine its potential as a novel treatment for smoking cessation.

Disclosure: A. Pushparaj: None. **C. Hamani:** Part 1: Consultant and honararia from St. Jude Medical., Part 3: Consultant and honararia from St. Jude Medical. **J. Nobrega:** None. **B. Le Foll:** Part 1: Salary support from Pfizer Consulting with Richter Pharmaceuticals, Part 2: Pfizer salary support, Part 3: Pfizer salary support, Part 4: Pfizer.

18. Prazosin Blocks Development of Ethanol, Morphine and Cocaine Conditioned Place Preference in Mice

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Background: Modulation of ventral tegmental area (VTA) dopaminergic neurons by norepinephrine at alpha-1 adrenoreceptors (AR) may mediate rewarding effects of drugs. Peripheral administration of the alpha-1 AR antagonist prazosin reduced heroin self-administration and blocked acquisition of morphine conditioned place preference (CPP).

Methods: Effects of central administration of prazosin at VTA on acquisition of ethanol, morphine and cocaine CPP was investigated in C57BL/6 mice, as was effect of prazosin on acquisition of lithium chloride (LiCl) conditioned place aversion. 26GA steel cannulae were implanted and aimed above VTA. CPP apparatus included two visually, tactically and odorously distinct chambers. Pretest was a 15-minute free run with time in each chamber. Drug conditioning began with injection of 0.9% saline and placement in one chamber ("saline chamber") for 30 minutes. Next, mice were infused with prazosin (1m/0.5ul/side) or vehicle (0.5ul/side) to VTA. Mice were then injected with morphine (5mg/kg), cocaine (10mg/kg), ethanol (2g/kg) or LiCl (3.0 meq) and placed in the other chamber (drug chamber) for 30 minutes times 3 days (4 days for ethanol). Next was a 15-minute post-conditioning test assessing place preference or aversion.

Results: Mice infused with vehicle but not prazosin developed a strong place preference to ethanol, morphine and cocaine. In contrast, both prazosin and vehicle groups demonstrated similar conditioned place aversion to LiCl, suggesting no prazosin effect on associative learning.

Discussion: These results support VTA alpha-1 AR mediation of positive reinforcing properties of ethanol, cocaine and morphine. They suggest potential efficacy of prazosin for substance use disorders.

Disclosure: M. Raskind: None. **V. Redila:** None. **V. Olson:** None.

19. Dopamine D1 Receptor Signaling Through Gαq: A Behavioral Assessment

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Background: Although dopamine D1-like receptors are typically thought of as coupling with Gαs or Gαolf to stimulate adenylyl cyclase activity, D1-like receptors are also capable of coupling to Gαq, resulting in phosphatidylinositol hydrolysis and intracellular calcium mobilization. It has been reported previously that this signaling pathway involves phosphorylation and activation of calcium/calmodulin-dependent protein kinase II (CaMKIIα), an important intracellular integrator of calcium signaling and regulator of synaptic transmission. Such functional selectivity in receptor-G protein signaling is regulated in brain region and cell-specific manners, however, the molecular determinants of these actions and their biological significance remain largely unknown. One recent and somewhat controversial hypothesis has suggested that dopaminergic modulation of Gaq signaling occurs through activation of a D1/D2 receptor hetero-oligomeric complex.
Methods: To further explore these relationships, we have used genetic models including D1, D2 and D5 receptor knockout mice, Gαq knockout mice and CaMKIIα knockin mice, in which the activating autophosphorylation site in CaMKIIα (threonine 286) is replaced with alanine and dopamine receptor antagonists to define the behavioral specificity of SKF83959, a reported D1 receptor-Gαq "biased" agonist. Mice were habituated to open field chambers (Med Associates) for 90 min sessions over multiple days. Photocell and video-based assessments of horizontal locomotor activity and orofacial stereotypies were performed. Data were analyzed by ANOVA.
Results: In wildtype mice, SKF83959 (1mg/kg) produced modest horizontal locomotor activation and a specific motor stereotypy involving facial grooming after peripheral injection. SKF83959-induced locomotor activity was absent in D1 receptor null mice but intact in D5 receptor knockouts, suggesting that the D1 receptor proper is the primary target for the behavioral effects of SKF83959. SKF83959-induced facial grooming also appears to be primarily mediated by the D1 receptor, although this effect was also partially blunted in D5 mutant mice. Gαq knockout mice also had greatly reduced locomotor activation following agonist exposure and expressed reduced baseline and SKF83959-induced facial grooming. Contrary to our hypotheses, however, SKF83959-induced behaviors were conserved in both D2 receptor knockout mice and CaMKIIα knockin mice, suggesting that these proteins are not necessary for SKF83959-mediated signaling or behaviors.
Discussion: These data suggest that current models and hypotheses of functional selectivity of the dopamine D1 receptor are inadequate. Our studies define complex receptor-G protein signaling in the regulation of brain signaling and behavior and may lead to the identification of new therapeutic targets for dopaminergic dysregulation.
Disclosure: G. Stanwood: None. **T. Saborido:** None. **R. Colbran:** None. **A. Frederick:** None.

20. A History of Cocaine Intake produces Persistent Reductions in the Functional Integrity of Glial Cells in Forebrain

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Background: Much of the research on the molecular adaptations resulting from cocaine exposure has centered on neuronal populations. Until recently, glial cells had been viewed only as support cells for neurons, but findings of their roles in regulating nerve impulse conduction, glutamate and GABA neurotransmission, maintaining the extracellular milieu, and their expression of many neurotransmitter receptors has made them a target for further research in the neurobiology of addiction. The present immunoblotting study was intended to examine a variety of glial cell subtype-specific proteins involved in the functional integrity of the forebrain, including the dorsal and ventral prefrontal cortex (dPFC and vPFC respectively) and the nucleus accumbens core and shell (NAc and NAs respectively).
Methods: Groups of rats were trained to lever-press for 0.25 mg/infusions of cocaine during 10 daily, 6-hr sessions. Control animals received daily 1-hr training to lever-press for saline. At 3 or 30 days following the last self-administration session, animals were subjected to a 2-hour test for cue-reinforced behavior then tissue was extracted for immunoblotting for a variety of markers of astrocytes and oligodendrocytes.
Results: Animals with a history of cocaine administration exhibited time-dependent increases in cocaine craving ($IV \times$ Withdrawal ANOVA, $p < .05$). Cocaine experience resulted in early and persistent decreases in vPFC myelin basic protein (MBP; $F(1,32) = 5.96$, $p = 0.021$), myelin oligodendrocyte glycoprotein (MOG; $F(1,35) = 7.67$, $p = 0.009$), the NG2(+) proteoglycan ($F(1,36) = 4.52$, $p = 0.04$) expression. Within the NAc, cocaine intake produced a persistent reduction in Cx43 expression ($F(1,48) = 7.836$, $p = 0.008$). No statistically significant changes were seen in expression levels within the dPFC or the NAs.
Discussion: A history of extended access to cocaine self-administration produced a time-dependent increase in cocaine craving. While none of the observed changes in our glial-specific markers exhibited time-dependent changes that coincided with behavior, the present data from rat are consistent with the human diffusion tensor imaging and postmortem gene expression findings indicating perturbations in oligodendrocyte function in cocaine addicts. Given the role of myelin in normal neuronal functioning and survival, these data suggest a reduction in the functional integrity of oligodendrocytes as a potential mechanism underpinning the enduring PFC hypofunction reported in cocaine addicts. Unlike the PFC, we did not detect changes in myelin-related proteins within the accumbens, despite evidence from postmortem tissue of chronic cocaine addicts indicating decreases in accumbens MBP. However, consistent with earlier reports that subchronic (7 days) cocaine experience is sufficient to influence astrocytic markers within the accumbens, we observed a reduction in one connexin - Cx43 - within the NAc. Connexins are gap junction proteins that allow direct intracellular communication of small molecules. On astrocytes, Cx43 is located at the endfeet which wrap synapses, thus a reduction in the expression of this protein may well related to alterations in neurotransmission within this region following a history of cocaine exposure. It is noteworthy that we observed enduring cocaine-elicited reductions in markers of glial integrity in the fully mature brain. Recent attention in the field of addiction has implicated adolescence as a period of high vulnerability, which is a critical period for brain development, particularly glial maturation. Thus, future studies will examine the impact of a history of cocaine self-administration during adolescence upon indices of glial integrity within forebrain stuctures exhibiting anomalies in addiction.
Disclosure: S. Webb: Part 5: UCSB. **A. Sacramento:** Part 5: UCSB. **O. Ben-Shahar:** Part 5: UCSB. **T. Kippin:** Part 5: UCSB. **K. Szumlinski:** Part 5: UCSB.

21. Interaction between Ethanol and Nicotine within the Mesolimbic Dopamine System: Evidence for Synergy and Distinct Alterations in Gene Expression

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Background: The vast majority of alcoholics smoke (> 90%), and individual diagnosed with nicotine dependence are over 4.5 times

more likely to be alcoholics than the general population. Clinical evidence suggests a clear comorbid relationship between ethanol (EtOH) and nicotine use/abuse, where the intake of one drug stimulates the use or increases the amount of intake of the other drug. The current experiments examined the interaction between EtOH and nicotine within the mesolimbic dopamine system, a key neurocircuit for drug reward.
Methods: The initial experiment examined intracranial self-administration (ICSA) of sub-threshold concentrations of EtOH (50 mg%) and nicotine (1 μM) or co-administration of EtOH and nicotine (25 or 50 mg% EtOH + 0.25, 0.5, or 1.0 μM nicotine) into the posterior VTA. To determine the consequence of EtOH, nicotine or the combination of both in the pVTA on a neural circuit of the reward pathway, TaqMan® low density RT-PCR arrays (TLDA) were designed to simultaneously assay expression of 85 GABA/Glutamate related genes and five additional genes associated with neuronal plasticity. In the first TLDA experiment, rats received experimenter controlled equivalent microinjections of aCSF, EtOH (50 mg%), nicotine (1 μM) or EtOH + Nic (50 mg% + 1 μM). In the last experiment rats self-administered aCSF, EtOH (50 mg%), nicotine (1 μM) or EtOH + Nic (50 mg% + 1 μM) directly into the posterior VTA for 5 sessions. Three hours after injections or the last self administration study rats were sacrificed and the accumbens shell (AcbSh) and posterior VTA (both ipsilateral to the site of drug administration) were removed and processed for TLDA analysis.
Results: The ICSA data set indicated that low concentrations of EtOH and nicotine did not support self-administration, but combining EtOH + Nic at these concentrations readily supported the development of self-administration of these compounds directly into the posterior VTA. The results from the experimenter controlled drug exposure study indicated that co-administration of EtOH + Nic into the posterior VTA produced a cascade of altered gene expression in the AcbSh that was not observed in rats administered only EtOH or nicotine. Interestingly, microinjection of EtOH + Nic into the posterior VTA resulted significant increases in BDNF gene expression and reductions in GDNF in the AcbSh (compared to aCSF), all other groups were equivalent, suggesting induction of neuronal plasticity. Additionally the EtOH + Nic microinjections into the pVTA, compared to aCSF, resulted in significant changes in the GABA and Glutamate systems, highlighted by significant decreased expression of GABA synthesis genes (GAD1 and GAD2) and significant increase in GABA B receptor 2 and metabotropic glutamate 2 receptors, and alterations in glutamate transporters. Major differences in the pattern and number of self-infusions in the 2nd experiment forced an examination between aCSF and EtOH + Nic groups only. ICSA of EtOH + Nic directly into the pVTA, compared to aCSF, resulted in a reduced gene expression of multiple glutamate and GABA receptors/subunits, alterations in GABA and glutamate trafficking/anchoring genes and transporters, in the pVTA.
Discussion: The data indicated that EtOH and nicotine within the posterior VTA can synergistically act to support the development of self-administration (reward). In addition, co-administration/injection of EtOH + Nic into the posterior VTA resulted in unique alterations in gene expression in the AcbSh and pVTA. Many of the altered genes are associated with the development of sensitization, drug-induced neuroadaptations, and drug addiction (i.e., BDNF, GDNF, Homer3, $GABA_{B2}$). The results provide an interesting foundation for future studies to examine the interactions of EtOH and nicotine within the mesolimbic dopamine, how these interactions promote co-morbid drug addiction/use, and the potential development of pharmacotherapeutics for the treatment of co-abuse.
Disclosure: W. Truitt: None. **S. Hauser:** None. **G. Deehan:** None. **Z. Ding:** None. **A. Shekhar:** Part 4: Research grants from J&J and Eli Lilly. **W. McBride:** None. **Z. Rodd:** None.

22. A Premorbid Depressive State Facilitates the Development of Dependence-like Cocaine Intake in Rats with Extended Access
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Background: A strong comorbidity between psychiatric disorders and drug dependence has been well documented withdepression as one of the most prevalent disorders in dependence on various drugs. However, a causal relationship between psychiatric disorders and drug addiction is not yet clear. The present study tested the hypothesis that pre-morbid depression facilitates the development of cocaine dependence using a rodent model of cocaine self-administration with extended access and a rodent model of depression.
Methods: Wistar Kyoto (WKY, n = 16) and Wistar rats (n = 16) were trained to self-administer cocaine (0.5 mg/kg/injection) with daily one-hour access under a fixed-ratio schedule (baseline session). After 13 sessions, the dose-response function of cocaine self-administration was determined. Then, the rats were again allowed to self-administer 0.5 mg/kg/injection of cocaine for seven more days, and pre-pulse inhibition (PPI) of startle reflex to acoustic stimulus in rats was determined at 22-hour withdrawal point from the last cocaine self-administration. Then the rats were divided into two groups per strain based on cocaine intake on the last baseline session. One group of each strain self-administered cocaine with one-hour access (short access, ShA) whereas the other group did so with six-hour access (long access, LgA), after which PPI and forced swimming behaviors were measured.
Results: All rats acquired cocaine self-administration. However, Wistar rats showed a faster rate of acquisition than WKY rats. Additionally, cocaine intake in WKY rats was lower than that of Wistar rats showing the downward shifted dose-response function of cocaine self-administration. With extended access to cocaine, both strains of LgA rats increased their cocaine intake. However, WKY LgA rats showed a faster rate of increase in cocaine intake than Wistar LgA rats. Interestingly, with protracted exposure to cocaine, cocaine intake also increased in WKY ShA rats, but not in Wistar ShA rats. Wistar rats exhibited a higher level of baseline startle reflex than WKY rats. However, there was no difference in the magnitude of prepulse inhibition (PPI) of startle reflex between two strains of rats. Cocaine self-administration did not alter PPI over the course of the study in both strains of rats. Consistent with the literature, WKY rats spent greater time in immobility and less time in swimming in a forced swim test (FST), suggestive of depressive-like state, compared with Wistar rats. When measured after 19 days of cocaine self-administration with extended access, both strains of LgA rats showed increased depressive-like behaviors in FST compared with ShA rats, however, the strain difference within each access condition remained unaltered.
Discussion: In support of the hypothesis, the results show that a pre-morbid depression-like state induces a faster rate of escalation of cocaine intake in rats with extended access. The data also suggest that extended access to cocaine self-administration induces depressive-like states in rats and that the pre-morbid state of depression may exacerbate drug withdrawal symptoms from cocaine self-administration with extended access.
Disclosure: S. Wee: None. **G. Koob:** None.

23. Valproic Acid reverses Age-Related decreases in Haloperidol-Induced c-Fos Expression in the Nucleus Accumbens
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Background: Antipsychotic drugs are widely prescribed to elderly patients for the treatment of a variety of neuropsychiatric

syndromes, especially the behavioral disturbance associated with dementia. However, recent studies suggest that these drugs are far less effective in the elderly than anticipated. While the mechanisms of age-related changes in the pharmacodynamics of antipsychotic drugs are not well understood, age-related epigenetic alterations may be involved. Antipsychotics activate immediate-early gene family, inducing *c-fos* expression in specific brain regions. Drug-induced variations in c-Fos expression across different brain regions have been associated with drug efficacy and extrapyramidal side effects. Recent studies suggest that valproic acid (VPA), an epigenetic agent that inhibits histone deacetylases, may influence various forms of epigenetic alternations in gene expression. However, whether VPA can improve the therapeutic effects of antipsychotic drugs by influencing epigenetic processes in the aging population is not known.

Methods: Twenty-four months old (old) and 3 months old (young) C57/B6 mice were used in this study. *Experiment 1*- To evaluate behavioral and cellular responses to haloperidol in the two groups of mice, a 0.01 mg/kg dose was administered (i.p.) once a day for 7 consecutive days. Thirty minutes after injection on the final day, spontaneous alteration and catalepsy tests were conducted. After behavioral testing, the mice were sacrificed and c-Fos expression patterns in the different brain areas including the nucleus accumbens shell (NAs) and stratum were examined by immunohistochemisty staining. *Experiment 2*- To elucidate the effects of VPA on c-Fos expression in old mice compared young mice, combined treatment of VPA with haloperidol (VPA + HAL) was administered. VPA (2.5mg/kg) was injected (i.p), twice a day for 6 consecutive days. On day 7, mice received the same dose of VPA and a single dose of haloperidol 0.1 mg/kg. Ninety minutes after the last drug injection, behavioral tests and c-Fos expression patterns in different brain areas were evaluated.

Results: *Experiment 1*. Spontaneous alteration showed a significant reduction in correct alternations (%) in old mice as compared to young mice ($p < 0.05$) and haloperidol administration (0.01mg/kg) further impaired spontaneous alternation in old mice ($p < 0.001$), but not in young mice. Administration of haloperidol also produced more cataleptic episodes in old miceas compared to young mice ($p < 0.05$). In addition, the duration of cataleptic episodes was increased in the old mice relative to young mice ($p < 0.001$). Finally, haloperidol-induced c-Fos expression in the NAs was reduced in old mice as compared to young mice ($p < 0.001$). *Experiment 2*. After pretreatment with VPA (as compared to vehicle), haloperidol-induced c-Fos expression was increased in old mice ($p < 0.0037$), but not in young mice.

Discussion: Increased haloperidol-induced impairments in spontaneous alternation, catalepsy and decreased c-Fos expression in the NAs was observed in old mice as compared to young mice. Pretreatment with VPA reversed age-related reductions in haloperidol-induced c-Fos expression in the NAs, These data suggest that VPA help to overcome at least some age-related changes in antipsychotic drug responsiveness. Further experiments are underway to examine additional behavioral measures related to clinical efficacy, multiple doses of VPA, and the cellular mechanisms by which VPA might exert such effects (e.g. histone modification).

Disclosure: J. Montalvo-Ortiz: None. **K. Murphy :** None. **J. Csernansky:** Part 1: Serves on data monitoring committees for Sanofi-Aventis and Eli Lilly. **H. Dong:** none.

24. 5-HT2AR and MGluR2 Play Opposing Roles in Regulating Anxiety

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Background: Anxiety and depression are among the most common psychiatric conditions, but we still lack a clear understanding of the anatomical and neurochemical circuitry necessary to improve treatment strategies. Clearly, limbic circuitry plays an important role in anxiety and fear but, more recently, the cortex has been shown to be a top-down modulator of anxiety processes, regulating the complex interplay between regions of interest including the hippocampus, and amygdala. We have previously shown that cortical 5-HT2A receptors are involved in anxiety-related behaviors including the light dark box performance, elevated plus maze and open field. We have also shown that in this cortical location, 5-HT2A receptors form heterodimeric complexes with a population of postsynaptic mGlu2 receptors. This heteromeric complex is of critical importance to the mechanism of action of hallucinogenic drugs such as LSD, however the role played by this heteromeric complex in anxiety states has not been established.

Here we investigate the role of the mGlu2 receptor in modulating anxiety states with the goal of identifying the population of these receptors regulating anxiety and further clarifying the nature of the interaction between mGluR2 and 5-HT2AR.

Methods: Open field: Wild-type and knockout mice were placed in a chamber with Plexiglas walls (16×16 inches) and locomotor activity including ambulation time, distance, rearing and thigmotaxis were measured using Kinder Scientific MotorMonitor Software. Light-Dark box: MedAssociates Open Field chambers were fitted with opaque inserts which cover half of the open field arena. Activity, including time spent and distance covered in the light and dark chambers were assessed in addition to time taken to enter the light field. Elevated plus maze: WT and KO mice were placed in the center of an elevated plus maze and behavior was recorded and scored for 2×5 minutes bins. Statistical comparisons were performed using Statview.

Results: In support of our previous findings we found that 5-HT2A receptor knockout mice showed reduced anxiety in traditional anxiety paradigms including the open field, light-dark boxes and the elevated plus maze. 5-HT2A receptor knockout mice spent significantly more time in the center of an open field arena, increased time in the light side of the light dark boxes as well as increased time spent in the open arms of the elevated plus maze. Restoring expression of the 5-HT2A receptors in cortical locations restores anxiety to levels similar to those observed in wild-type mice. Using a novel line of mGluR2 knockout mice, which possess the same capacity for expression to be selectively restored, we see that in the absence of mGluR2 mice engage in significantly more thigmotaxis than WT mice. These mice also fail to show an increase in rearing/exploratory behavior over the first two bins (15 min each) of exploratory open field activity, which is observed in wildtype mice, however this result did not reach statistical significance at the current sample size. At the time of writing, the elevated plus maze, light-dark box analysis, and cortical rescue were in progress.

Discussion: In the cortex, the metabotropic glutamate receptor 2 and the serotonin 2A receptor have been shown to function as a heteromeric complex, to mediate the effects of hallucinogens. Here, our data suggests that they may act in opposition to each other in anxiety-like behavioural paradigms. However, mGluR2 expression is not only observed in the cortex. It is also strongly expressed presynaptically in the hippocampus, and amygdala – both known to be critical to anxiety states. Thus, it remains to be established whether the receptor populations responsible for modulating the response to anxiogenic stimuli are co-localized (and possibly heterodimerising) or whether these populations reside in divergent neural locales, working together to control anxiety from a network level.

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25. Neurotropic Viruses and Induced Pluripotent Stem Cells: Tools for Probing Neurodevelopment

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Background: Background: Recent advances in cell culture technologies for neural differentiation of human induced pluripotent stem cells (iPSC) have provided an unprecedented opportunity to study human brain development and model neurodegenerative and neurodevelopmental diseases.We have initiated neurodevelopmental studies using human cytomegalovirus (HCMV) as a tool to perturb normal neural differentiation. HCMV infects neural stem cells and neuroprogenitor cells localized in ventricular and subventricular zones. It is a major cause of prenatal encephalitis and mental retardation. HCMV has been investigated using neurospheres prepared using forebrain tissues from fetal abortuses. These models provide important information, but have obvious limitations. Hence we have investigated the effects of HCMV infection on human iPSC-derived neural stem cells, neuropregenitor cells and neurons.
Methods: iPSCs were generated from adult human fibroblasts obtained through adult skin biopsies. We developed a novel method for neural differentiation of iPS cells. The stages of differentiation include neural rosettes composed of neural stem cells, neurospheres, neuroprogenitor cells, and neurons. All these lineages were infected with human cytomegalovirus (HCMV) at multiplicity of infection (MOI = 3). Functional competency of neurons was confirmed by live imaging of intracellular calcium influx.
Results: Our result suggests that (i) iPS cells are not permissive to HCMV infection; (ii) HCMV infected neural stem cells differentiate into neurons that undergo degeneration; (iii) neural differentiation is inhibited in infected cells where the viral DNA replication is increased; (iv) neuroprogenitor cells are fully permissive for HCMV infection; (v)most iPS-derived neurons are not permissive to HCMV infection; and (vi) infected neurons have impaired calcium influx in response to glutamate. In fact, no evoked intracellular calcium increase was detected in HCMV-infected neurons after glutamate administration as observed in control neurons.
Discussion: Our approach offers powerful cellular models to investigate the effect of neurotropic viral agents on human neurodevelopment at molecular and cellular levels. Combined with high throughput, unbiased RNA sequencing technologies, our model will enable comprehensive gene expression studies under carefully controlled conditions.
Disclosure: L. D'Aiuto: None. **B. Heath:** None. **A. Watson:** None. **B. Mikhil:** None. **R. Di Maio:** None. **G. Raimondi:** None. **V. Nimgaonkar:** None.

26. AMPA Receptor Mediated Mitogen-Activated Protein Kinase Signaling in Oligodendrocytes

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Background: Recent evidence demonstrates cerebral white matter alterations in schizophrenia such as changes in myelin related proteins including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP). Additionally, changes in oligodendrocyte-specific transcription factors have been demonstrated in schizophrenia. Myelination in the brain continues throughout late adolescence which not only correlates with the time period for the onset of schizophrenia, but is most evident in the frontal and temporal lobes, areas of the brain associated with schizophrenia. The signaling mechanisms responsible for myelination are not known. Vesicular release of glutamate from axons induces glutamate receptor mediated currents in postsynaptic oligodendrocyte progenitor cells, underscoring the importance of studying glutamate as a signaling molecule during myelination. Our data show that glutamatergic signaling in oligodendrocytes activates mitogen-activated protein kinase signaling cascades that cause changes in transcription of myelin-related proteins.
Methods: Primary mixed glial cultures containing astrocytes, oligodendrocytes, and microglia were isolated from the forebrains of 2-day old rats using a differential detachment method. Microglia were separated by shaking the mixed glial-containing flasks and the resulting oligodendrocyte suspension was plated onto 24 well plates. Typically, these cultures are 95% OLs, 1–2% astrocytes, and 1–2% microglia. After treatment, cell lysates were evaluated for total and phosphorylated protein by western immunoblotting. Actin was evaluated as a loading control.
Results: Our data demonstrate that glutamate activates p38 and ERK mitogen-activated protein kinase (MAPK) signaling cascades in oligodendrocytes that are blocked by antagonists of the á-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptor. We provide evidence that glutamate receptor activation leads to enhancement of mRNA and protein expression of myelin basic protein (MBP) that is blocked by AMPAR antagonists and inhibitors of p38 and ERK MAPKs. Glutamate stimulation causes phosphorylation of cAMP response element-binding protein (CREB) that is blocked by the AMPAR antagonist (NBQX), the p38 inhibitor (SB203580), and the ERK inhibitor (U0126). Transcription factor analysis of the MBP promoter region identified discrete nucleotide regions with high homology to P-CREB transcription factor binding sites suggesting that its activation by MAPK signaling may regulate MBP expression.
Discussion: These data indicate that glutamatergic signaling is necessary for myelination. Hypofunction of glutamatergic signaling in neurons has been implicated in schizophrenia suggesting that glutamatergic signaling in the cerebral white matter may also be disrupted. Taken together these data suggest that an alteration in glutamatergic signaling may underlie the white matter changes observed in schizophrenia.
Disclosure: T. DeSilva: None. **B. Baker:** None. **A. Funk:** None.

27. Proinflammatory Gene Expression in Response to Stressors Differs across Brain Regions and is Programmed by Maternal Diet

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Background: Intrauterine programming is increasingly recognized as profoundly influencing offspring psychological and physiological outcomes. Both maternal over-nutrition and maternal under-nutrition, resulting in offspring which are respectively large for gestational age (LGA) or small for gestational age (SGA), are associated with adverse neurobehavioral outcomes, including increased susceptibility to mood disorders, addiction, and neurodevelopmental disorders. Our laboratory has recently demonstrated that both LGA and SGA offspring demonstrate profound changes in reward function and dopamine-related gene expression in mesocorticolimbic structures, which may provide a background of increased susceptibility to psychological disorder. However, less is known about how gestational growth influences functioning of the mesocorticolimbic system in response to stress, which can prompt the onset of psychological disorder. Central stress-induced proinflammatory gene expression is of increasing interest as an underexplored signaling mechanism involved in the regulation of stress responses. We are currently examining

proinflammatory gene expression in mesocorticolimbic structures of LGA and SGA mice in response to stress.
Methods: Male and female LGA, SGA, and control C57xDBA F1 mice at postnatal day 60 were sacrificed at baseline or 2 hours after onset of either 15 minute restraint (psychological stress) or intraperitoneal lipopolysaccharide (LPS; physical/immunological stress). Tissue was preserved in RNAlater and the prefrontal cortex, nucleus accumbens, hypothalamus, central amygdala, basolateral amygdala, and ventral tegmental area were dissected. Standard qRT-PCR was conducted in the hypothalamus and nucleus accumbens examining induction of proinflammatory mediators tumor necrosis factor alpha (TNF), cyclooxygenase-2 (COX-2), and inhibitor of kappa-B alpha (IkBa) mRNA. We are currently conducting high-throughput qRT-PCR on all of the above named regions on a spectrum of cytokines and proinflammatory mediators, as well as epigenetic modifiers.
Results: Our current results indicate specific interactions between stress and maternal diet at baseline and especially induced by the two stressors. However, these are mediated by significant sex differences, and regional specificity of gene expression. In the hypothalamus, both LGA and SGA males and females demonstrate suppressed basal COX-2 and IkBa transcription. However, in response to either LPS or restraint, SGA and LGA males demonstrate a hyperactivation of these genes in the hypothalamus. In contrast, in the nucleus accumbens, both LGA males and females demonstrate hypoactive TNF and COX-2 response to both stressors, but no significant effects were observed at baseline or in SGA animals.
Discussion: Abnormally small or large birthweights, which are influenced by multiple factors during gestation including maternal diet, are associated with increased risk for subsequent psychological disorders and can be considered biomarkers for these risks. In our models of LGA and SGA, we have identified altered proinflammatory gene expression and a differential neuroinflammatory response to stressors as common potential contributors to neurobehavioral vulnerability in offspring.
Disclosure: N. Grissom: None. **R. George:** None. **T. Reyes:** None.

28. Neurexin 1 (NRXN1) Gene Expression across the Human Lifespan: Implications for Neurodevelopment and Schizophrenia

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Background: Deletions and rare polymorphisms in NRXN1 are associated with neurodevelopmental disorders including autism and schizophrenia. Mutations in schizophrenia primarily affect the NRXN1- α promoter and 5′ exons. NRXN1 (2p16.3), a presynaptic cell adhesion molecule and receptor that plays critical roles in synaptogenesis, encodes two major isoforms, NRXN1-α and NRXN1-â. Given the association of NRXN1 with neurodevelopmental disorders, we characterized the developmental trajectory of NRXN1-β and β expression in normal human brain across the lifespan and in patients with schizophrenia.
Methods: Postmortem brains were collected at the Clinical Brain Disorders Branch, NIMH. Fetal specimens were provided by NICHD. Dorsal lateral prefrontal cortex (DLPFC) was available from 247 normal individuals (0-80 years) and 113 schizophrenia patients. Fetal PFC was derived from 42 individuals (14-39 weeks). Total NRXN1 (α and β); NRXN1-α and NRXN1-β mRNA were measured using Quantitative RT-PCR.
Results: Linear regression revealed significant effects of age on NRXN1-α ($P = 1.80E^{14}$); NRXN1-β ($P = 4.61 \times 10^{-15}$) and total NRXN1 ($P = 4.08E^{23}$) expression across the normal postnatal lifespan. NRXN1 expression was highest in early neurodevelopment, peaking around 2-3 years and steadily declining. In the fetal brain, NRXN1-α ($P = 0.003$); NRXN1-β ($P < 0.0001$) and total NRXN1 ($P = 1.80E^{10}$) increased significantly with gestational age. The expression of both isoforms peaked at 39 weeks gestational age.NRXN1-α ($P = 4.08E^{34}$), but not NRXN1-β, was more abundant in the fetal compared with postnatal brain, suggesting a significant role of NRXN1-α in early brain development. In patients with schizophrenia, a significant increase in NRXN1-β expression, and a significant decrease in the ratio of NRXN1-α/NRXN1-β was observed in DLPFC compared to age-matched controls.
Discussion: Our data provide insight into brain NRXN1 expression profiles across the human lifespan and demonstrate that expression is highest during critical periods of synaptic development, consistent with the association of NRXN1 with neurodevelopmental disorders. Furthermore, imbalances in the expression of NRXN1 isoforms are observed in patients with schizophrenia.
Disclosure: A. Jenkins: None. **Y. Wang:** None. **T. Hyde:** None. **J. Kleinman:** None. **A. Law:** None.

29. ZNF804a Regulates Transcription of the Schizophrenia-Associated Genes PRSS16, COMT, NRG1, PDE4B, and DRD2

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Background: Schizophrenia (SZ) is a heritable disorder having no single gene or factor that accounts for a majority of cases. Many gene variants are associated with SZ and an abundance of these genes are clustered in signaling pathways important to neurodevelopment, synaptic transmission and immune function. An emerging hypothesis to explain SZ etiology suggests that epistatic interactions between multiple susceptibility genes all converge onto a set of biological networks important for SZ pathology. If SZ susceptibility genes are closely connected to a common disease pathway, then controlling the transcription of genes within this network would be critical for proper biological function. Therefore, we hypothesized that transcription factors associated with SZ may be central to a transcriptional network that regulates the expression of other SZ susceptibility genes. ZNF804a was identified by a genome-wide association study (GWAS) in which a single-polymorphism (SNP) in ZNF804a reached near genome wide statistical significance for association with SZ and bipolar disorder. Although the molecular function of ZNF804a is unknown, the amino acid sequence is predicted to contain a C2H2-type zinc-finger domain and suggests ZNF804a plays a role in DNA binding and transcription. Although ZNF804a is a relatively strong candidate susceptibility gene, the molecular mechanism responsible for enhancing risk for psychosis remains unknown.
Methods: To determine if ZNF804a regulates transcription of a network of SZ susceptibility genes, we asked whether overexpression of ZNF804a in E11 rat forebrain neural progenitor cells would alter transcription of the top 38 SZ-associated genes highlighted on SZgene.org. We epitope-tagged a human ZNF804a clone and subcloned it into a plasmid containing a constitutive promoter (pCAG) creating pCAG-ZNF804a. Following a 24 hour tranfection in culture, we isolated RNA or chromatin to perform quantitative real-time PCR (qRT-PCR) or chromatin immunoprecipitation (ChIP), respectively.
Results: Overexpression of ZNF804a in cortical progenitor cells resulted in a significant alteration in transcript levels for 5 genes out of the 38 genes tested. We observed a significant increase in transcript levels, relative to GFP transfected controls, for three genes (Figure 1B; NRG1 2.19 ± 0.4, COMT 3.14 ± 0.75, and PRSS16 4.64 ± 0.2; all $p < 0.05$; $n = 5$) and a statistically significant decrease in transcript levels for two genes (Figure 1C; PDE4B -3.43 ± 0.23 and DRD2 -3.17 ± 0.43; all $p < 0.05$; $n = 5$). These results indicate that ZNF804a can modulate the transcription of several potential

SZ susceptibility genes, and suggests that the level of ZNF804a expression may coordinately alter the expression levels of a network of SZ susceptibility genes. We next performed ChIP to determine whether ZNF804a directly interacts with promoter regions of the 5 regulated genes indentified in the qRT-PCR assays. Chromatin immunoprecipitated for ZNF804a-myc was enriched for regions within the PRSS16 ($26.2 \pm 0.4\%$, $p < 0.05$; $n = 4$) and COMT ($20.2 \pm 0.2\%$, $p < 0.05$; $n = 4$) promoters. Furthermore, the promoter regions that immunoprecipitated with ZNF804a contained predicted zinc-finger interacting motifs (PRSS16 GGCG; COMT GGCGG). These results indicate that ZNF804a directly interacts with promoter regions for PRSS16 and COMT and is consistent with a direct positive action of ZNF804a on PRSS16 and COMT transcription.
Discussion: This study identifies several putative SZ-associated genes that are transcriptionally co-regulated by ZNF804a. SNPs in ZNF804a are associated with altered ZNF804a transcript levels, and therefore altered expression levels are a potential molecular mechanism to explain the association of ZNF804a with psychosis. We show that the genes regulated by ZNF804a encode proteins within four different pathways previously linked to SZ: dopamine signaling (COMT and DRD2), synapse development (NRG1), intracellular signaling (PDE4B) and immune function (PRSS16). Our results suggest that expression of ZNF804a directly links these diverse pathways, and more generally, provides additional evidence that a functionally interconnected genetic network is linked to SZ- susceptibility.
Disclosure: M. Girgenti: None. **J. LoTurco:** None. **B. Maher:** None.

30. Adenosine A2A Receptor Mediated Signaling in the Dorsomedial Striatum in Mice Lacking ENT1 and Alcoholism

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Background: Mice lacking an ethanol-sensitive adenosine transporter (type 1 equilibrative nucleoside transporter; ENT1) exhibit reduced ataxic and hypnotic effects to acute ethanol exposure and consume more ethanol than wild-type mice (Nature Neuroscience, 7:855-861, 2004). Our recent study revealed that decreased intracellular PKCgamma-neurogranin (Nrgn)-Ca^{2+}-calmodulin dependent protein kinase type II (CaMKII) signaling and CREB activity in the nucleus accumbens (NAc) might contribute to increase ethanol drinking in mice lacking ENT1 (Biological Psychiatry, 69:1043-1051, 2011).
Methods: We examined the signaling molecules using an iTRAQ proteomic method. We performed functional proteomic and ethanol drinking experiments to examine adenosine A2A receptor (A2AR)-regulated NMDA glutamate signaling in dorsal medial striatum (DMS) in ENT1 null mice.
Results: We identified down-regulation of PKCgamma-Nrgn-CaMKII-CREB activity in the DMS similar to the NAc, whereas no changes were observed in the dorsal lateral striatum (DLS). Interestingly, we found a DMS specific reduction of an active form of PKA, which was not altered in the NAc. Since adenosine levels are decreased in ENT1 null mice, we examined whether dampened A2AR signaling in response to decreased extracellular adenosine levels is causally related to decreased PKA activity. Moreover, decreased PKA-driven phosphorylation of NMDAR may be correlated with decreased PKCgamma-Nrgn-CaMKII-CREB activity. We found that A2AR antagonist (ZM241385) treatment significantly decreases phosphorylation of PKA in the DMS of wild-type mice to a level similar to that of ENT1 null mice. In addition, ZM241385 treatment also decreased both PKCgamma and CaMKII activity in wild-type mice, indicating that A2AR may play an important role in NMDAR-mediated PKCgamma-Nrgn-CaMKII signaling. In addition, ZM241385 treatment effectively increased ethanol drinking in wild-type mice.
Discussion: Our findings suggest that decreased A2AR activity may contribute to excessive ethanol drinking through similar signaling mechanism identified in ENT1 null mice. Since the DMS plays an essential role in transition from goal-direct to habitual behaviors in ethanol drinking behavior, adenosine A2AR signaling in the DMS might be an important therapeutic target for alcohol use disorders.
Disclosure: H. Nam: None. **D. Hinton:** None. **S. Choi:** None. **N. Kang:** None. **D. Choi:** None.

31. MicroRNA-132 drives Compulsive Cocaine Use through Nuclear Factor-κB Signaling

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Background: MicroRNAs (miRNAs) are small noncoding RNA transcripts expressed throughout brain that regulate neuronal gene expression at the post-transcriptional level. MicroRNA-132 (miR-132) is a CREB-regulated miRNA that is induced by neuronal activity and neurotrophins, and plays a role in regulating neuronal morphology and cellular excitability. Furthermore, it is an activity-dependent microRNA in vivo, and may contribute to the long-lasting protein changes required for experience-dependent neuronal plasticity. Interestingly, NF-κB (nuclear factor-êB) can potentially regulate the expression of genes governing changes in synaptic plasticity and cognitive functions, and it has been proposed its role as a critical modulator of the homeostatic interplay between inhibitory and excitatory neuronal function. Cocaine is known to increase NF-κB signaling in striatum, and cocaine addiction is commonly viewed as a disorder of neuroplasticity. We tested the potential involvement of miR-132 in cocaine addiction, and the role for NF-êB in the actions of miR-132.
Methods: Male Wistar rats (Charles River Laboratories, Raleigh, NC) weighing 300-320 g were surgically prepared with silastic catheters in the jugular vein and trained to respond on an "active" lever for food pellets (45 mg; 60 min sessions) under a fixed ratio 5 time-out 20 sec (FR5TO20) schedule of reinforcement. Rats then responded for cocaine on the FR5TO20 sec reinforcement schedule during 1 h daily testing sessions for at least 7 consecutive days. Cocaine hydrochloride was dissolved in sterile saline solution (0.9% w/v). Each cocaine infusion earned resulted in the delivery of 0.5 mg/kg/infusion cocaine (0.1 ml injection volume delivered over 4-sec), and initiated a 20-sec time-out period signaled by a light cue located above the active lever during which responding on the lever was without consequence. In all cases, a control group of rats were surgically prepared with jugular catheters and trained to respond for food reinforcement, but remained cocaine-naïve for the duration of the experiment. To determine the cocaine dose-response curve, the unit dose of cocaine available for self-administration was adjusted upward or downward during 3 hour testing sessions every other day between regular 6 h self administration sessions. Doses of cocaine were tested once, and in the following order: 0.5, 0.0625, 0.25, 0.125, and 0 mg/kg/infusion.
Results: We identified a key role for miR-132 in the dorsal striatum in the escalating cocaine intake seen in rats with extended access to the drug, a process that mimics the increasingly uncontrolled cocaine use seen in human addicts.Striatal miR-132 dramatically increases responsiveness to the motivational properties of cocaine by significantly amplifying the stimulatory effects of the drug on NF-κB signaling. We show that this action occurs through miR-132-enhanced PKCζ-Raf activity, resulting in increased expression of Rho kinase (ROCK), which we show to be an important activator of NF-κB signaling.

Discussion: These data suggest that miR-132 in dorsal striatum may play an important role in regulating vulnerability to cocaine addiction, and that this miRNA acts by amplifying the inflammatory regulator NF-κB.
Disclosure: H. Im: Part 4: F32 NRSA post doctoral fellowship, NIDA, NIH Young Investigator Award, NARSAD. **P. Bali:** Part 2: Yes. **P. Kenny:** Part 2: Yes, Part 4: Supported by a grant from the National Institute on Drug Abuse (NIDA) to P.J.K. (DA025983).

32. Chronic Wheel Running reduces Compulsive Methamphetamine Drug Seeking

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Background: One of the few interventions for psychostimulant addiction is physical activity. Voluntary wheel running in rodents can model high level of sustained physical activity and reduce motivation and escalation to cocaine self-administration, suggesting that wheel running modulates drug reinforcement by altering the neuroplasticity of the reward pathway. Hippocampal neurogenesis is implicated in the neuroplasticity of drug self-administration and wheel running, suggesting important implication for addiction. Here we are exploring the role of hippocampal neurogenesis underlying continued wheel running-induced reduction in acquisition and escalation of methamphetamine self-administration.
Methods: Adult male Wistar rats performed wheel running activity in their home cage for 6 weeks prior to surgery for intravenous catheters followed by intravenous methamphetamine self-administration in separate operant chambers. Rats were either withdrawn from running wheels or continued wheel running during self-administration days (0.05 mg/kg, i.v., FR1 1hr/day for 10 days followed by FR1 6hr/day for 22 days) and self-administration and running behaviors were monitored separately. Statistical analyses were performed by One-way or Two-way ANOVA with group as one factor and session numbers as within-subjects factor, followed by Fisher's Least Significant Difference *post hoc* test.
Results: Rats that continued running during self-administration self-administered significantly less methamphetamine than sedentary controls and wheel-withdrawn animals during the acquisition and escalation phase. In parallel, running wheel behavior was reduced after methamphetamine self-administration. Wheel-withdrawn animals self-administered significantly higher methamphetamine than sedentary controls during acquisition, but demonstrated delayed escalation of methamphetamine intake compared to sedentary controls. Animals from all groups are having cell proliferation in the dentate gyrus of the hippocampus quantified using the mitotic marker bromodeoxyuridine.
Discussion: Our findings suggest that wheel running-induced neuroplastic effects are transient and continued wheel running is protective against compulsive methamphetamine intake.
Disclosure: A. Englemann: None. **M. Aparicio:** None. **C. McKay:** None. **A. Kim:** None. **J. Sobieraj:** None. **Y. Grant:** None. **S. Wee:** None. **G. Koob:** None. **C. Mandyam:** None.

33. Targeting Synaptic Actin Dynamics to Weaken Memories Driving Methamphetamine Seeking Behavior

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Background: Methamphetamine (METH) is a highly addictive substance and considered by many health care professionals to be the hardest addiction to treat. Exposure to environmental cues associated with previous drug use can serve as a powerful relapse trigger, causing the involuntary retrieval of deeply engrained memories that induce intense craving and redirect behavior towards obtaining METH.Identifying the mechanisms responsible for these powerful memories should lead to new therapeutic targets. Synaptic dynamics is one particularly promising avenue, as emerging evidence indicates that memory formation depends on structural and functional plasticity of dendritic spines at excitatory synapses. Actin polymerization is a critical player in driving this plasticity. We recently reported that myosin IIb, a non-muscle form of myosin, imparts a mechanical force on the spine actin cytoskeleton in response to synaptic stimulation. This action is critical for the stabilization of early phase LTP and memory formation.
Methods: In order to determine if structural and functional plasticity regulates METH-associated memories, we employed two animal models of context-induced METH seeking, conditioned place preference (CPP) and reinstatement of self-administration.
Results: rAAV shRNA-mediated knockdown of MyH10, the heavy chain of myosin IIb, in the basolateral amygdala (BLA) prevented to the development of a METH place preference ($F_{(1,18)} = uuu1.8$, $p > 0.05$). Arguably, the greater therapeutic benefit lies in disrupting the subsequent control these memories bear over drug seeking behavior. To explore myosin II's potential role in this later stage of addiction, we pharmacologically inhibited myosin II ATPase activity in the BLA with blebbistatin (blebb) prior to retrieval of METH CPP. Not only did blebb prevent retrieval within minutes of infusion ($F_{(1,17)} = 8.4$, $p \leq 0.001$), but it also prevented retrieval during several subsequent, daily retrieval sessions in which no blebb was administered ($F_{(1,17)} = 5.7$, $p \leq 0.01$). Using a single reinstatement training session, we confirmed that blebb had not accomplished this lasting disruption of drug seeking through damage of the BLA ($p \leq 0.01$). In light of this unexpectedly strong result of myosin II inhibition on drug seeking, we sought to confirm the effect by targeting a different, but related mechanism of synaptic dynamics, actin polymerization. Disruption of actin polymerization within the BLA with Latrunculin A (LatA) produced a similar, long-lasting disruption of drug seeking ($F_{(1,19)} = 2.2$, $p \leq 0.05$). METH CPP memories are powerful, as demonstrated by the absence of extinction in vehicle-treated control animals over six consecutive, non-reinforced test sessions. Interestingly, when given LatA prior to Test 7, these previously vehicle-treated controls showed a similar disruption of preference for the METH-paired compartment ($p > 0.05$). Unexpectedly, exposure to METH-paired stimuli is not required for successful LatA disruption of drug seeking. Animals that received intra-BLA infusions of LatA in their home cage twenty-four hours before a retrieval test also failed to show drug seeking behavior ($F_{(1,7)} = 1.7$, $p > 0.05$), suggesting actin dynamics supporting drug-associated memories may display unique 'behavior'. Finally, preliminary evidence indicates that intra-BLA infusion of LatA also successfully prevents context-induced reinstatement of METH seeking in a self-administration paradigm.
Discussion: These findings demonstrate that the regulation of actin dynamics in the BLA provides critical support for deeply engrained METH-associated memories and, when disrupted, exposure to reminders of METH no longer drive drug seeking behavior.
Disclosure: E. Young: None. **E. Griggs:** None. **G. Rumbaugh:** None. **C. Miller:** None.

34. Characterization of Trace Amine Associated Receptor 1 Signaling and its Differential Effects on Dopamine Transporter and Norepinephrine Transporter Internalization

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Background: Trace amine associated receptor 1 (TAAR1) is activated by a spectrum of agonists including biogenic amines

and amphetamine-like psychostimulant drugs. TAAR1 activation alters dopamine neuron firing rates and modulates dopamine transporter (DAT), norepinephrine transporter (NET) and serotonin transporter kinetics, suggesting that it is a potential therapeutic target for the treatment of psychiatric and addictive disorders. The present report characterizes TAAR1 modulation of signal transduction and DAT and NET internalization.
Methods: We used transfected HEK293 cells (TAAR1, DAT, DAT + TAAR1, NET, NET + TAAR1), and striatal and thalamic tissues from wild type (WT, C57BL/6 strain) and TAAR1 knockout (KO; derived by Wolinsky *et al*., 2007) mice for these studies. We used a 45-pathway Cignal Finder™ array (SABiosciences) to determine which cellular signaling pathways were activated upon TAAR1 stimulation, and specific antibodies to detect phospho-PKA and phospho-PKC by Western blot. DAT and NET internalization in transfected cells and brain synaptosomes were monitored by biotinylation/Western blotting with DAT and NET antibodies.
Results: 1. TAAR1 signals through both the cAMP/PKA and PKC/Ca + + pathways in vitro: Methamphetamine (METH, 1 microM), a potent TAAR1 agonist, selectively activated both the cAMP/PKA and PKC/Ca + + pathways in human, rhesus monkey and mouse TAAR1-transfected HEK293 cells, but not in untransfected HEK293 cells or in other transfected cell lines (e.g., DAT, NET) in the absence of TAAR1. 2. TAAR1 activation increases phosphorylation of both PKA and PKC: METH (1 microM/10 min) increased phosphorylation of both PKA and PKC in TAAR1-transfected HEK293 cells and in WT mouse synaptosomes isolated from striatum (DAT dense) and thalamus (NET dense), but not in untransfected HEK293 cells, in cell lines transected with DAT or NET in the absence of TAAR1, or in striatal or thalamic synaptosomes from TAAR1 KO mice. 3. TAAR1 triggers PKC-dependent DAT internalization: METH (1 microM) treatment induced time-dependent DAT internalization in TAAR1 + DAT-transfected HEK293 cells in vitro and WT mouse striatal synaptosomes ex vivo, but failed to do so in DAT-only-transfected HEK293 cells in vitro or in TAAR1 KO mouse striatal synaptosomes ex vivo. These findings were relevant in vivo: METH (1 or 10 mg/kg) i.p. administration in vivo induced a time-dependent DAT internalization in rapidly-excised striatum of WT mice, but not in TAAR1 KO mice. In further in vitro and ex vivo studies, DAT internalization in response to either the TAAR1 agonist METH (1 microM) or direct activation of PKC by a PKC activator (PMA; 1 microM) was blocked by a PKC inhibitor (Ro32-0432; 10 microM) and was unaffected by concurrent activation (8-Br-cAMP; 100 microM) or blockade (H89; 10 microM) of the PKA pathway. 4. Unlike DAT, PKA signaling modulates PKC-dependent NET internalization: In parallel experiments, METH did not induce NET internalization in NET- or TAAR1 + NET-transfected cells, or thalamic synaptosomes from WT or TAAR1 KO mice following ex vivo or in vivo administration. However, METH (1 microM) induced robust NET internalization when the PKA pathway was concurrently inhibited (H89; 10 microM) in TAAR1 + NET cells in vitro and in WT mouse thalamic synaptosomes ex vivo, but failed to do so in NET-only-transfected HEK293 cells in vitro or in TAAR1 KO mouse striatal synaptosomes ex vivo. Direct activation of the PKC/Ca + + pathway with PMA (1 microM) induced internalization of NET in all circumstances, whereas the direct activation of the cAMP/PKA pathway with 8-Br-cAMP (100 microM) had no effect on NET internalization. However, concurrent PKA activation by 8-Br-cAMP (100 microM) blocked PKC-driven NET internalization by PMA (1 microM).
Discussion: Together, these findings confirm the linkage of TAAR1 to both cAMP/PKA and PKC/Ca + + pathways, reveal that TAAR1 is a trigger that mediates PKC-dependent DAT internalization, and uncover a differential role for TAAR1 in modulating DAT vs. NET internalization. We show that PKA modulates PKC effects on NET trafficking but not DAT trafficking and that concurrent activation of both pathways by TAAR1 results in no NET internalization in response to METH. Collectively, these data highlight novel mechanisms by which TAAR1 affects catecholamine transporter mobility, and suggest that brain catecholamine signaling systems are modulated by METH and other TAAR1 agonists via previously unidentified mechanisms. The findings warrant assessment of TAAR1 compounds as therapeutics for neuropsychiatric and addictive disorders, in which catecholamine transporters and signaling are implicated.
Disclosure: G. Miller: None. **B. Madras:** None. **X. Zhihua:** None.

35. Chronic Intermittent Ethanol Exposure produces Brain Region Specific Alterations in Immunohistochemical Labeling of the Neuroactive Steroid Allopregnanolone during Withdrawal in C57BL/6J Mice

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Background: The GABAergic neuroactive steroid allopregnanolone ((3alpha,5alpha)-3-hydroxypregnan-20-one) has been studied during ethanol withdrawal in humans, rats and mice. Prior studies have shown decreases in human serum, but no change in rat or mouse cortex during the peak of ethanol withdrawal. Immunohistochemical detection of allopregnanolone in brain sections allows detailed analysis of the effects of chronic intermittent ethanol (CIE) exposure and withdrawal across brain regions. Because CIE exposure increases subsequent voluntary ethanol drinking, we examined brain regions known to influence this behavior.
Methods: Adult male C57BL/6J mice were exposed to the CIE model of ethanol dependence. Briefly, after establishing stable baseline drinking using a limited access (2 hr/day) 2-bottle choice (15% ethanol vs. water) paradigm, mice received four cycles of chronic intermittent exposure (16 hr/day × 4 days) to ethanol vapor (EtOH group) or air (CTL group) in inhalation chambers. Exposure cycles 1-3 were followed by a week of daily limited access drinking test sessions. All mice were sacrificed and perfused at 8 hr following the final exposure cycle. Free floating brain sections (40 microns; 3-4 sections/region) were immunostained and analyzed for each animal. A mean value for the vehicle group was calculated and compared to the values for each ethanol-exposed mouse. Data represent the mean ± SEM of % control values, n = 7-8 mice/group. Data were analyzed by two-way ANOVA followed by post-hoc tests.
Results: As expected, ethanol drinking (g/kg) significantly escalated in EtOH mice over successive test cycles compared to their own baseline levels of intake as well as the CTL mice. Immunohistochemical labeling with the allopregnanolone antibody was inhibited by allopregnanolone itself and 3alpha-hydroxyprogesterone, but no cross reactivity with tetrahydrodeoxycorticosterone was observed. CIE exposure produced region-specific effects on immunohistochemical detection of allopregnanolone levels across limbic brain regions. We observed increases in cellular allopregnanolone-like immunoreactivity in the prefrontal cortex (15.4 ± 3.2%, $p < 0.002$), bed nucleus of the stria terminalis (23.0 ± 6.0%, $p < 0.02$), ventral tegmental area (23.0 ± 8.8%, $p < 0.05$), nucleus accumbens (16.1 ± 2.9%, $p < 0.05$) and the medial aspect of the central nucleus of the amygdala (28.7 ± 9.1%, $p < 0.05$) in the EtOH mice. In contrast, we found a reduction in allopregnanolone-like immunoreactivity in the basolateral amygdala (23.6 ± 7.0%, $p < 0.05$), but no effect of CIE in the central aspect of the central nucleus of the amygdala or the lateral amygdala.
Discussion: These data suggest that specific adaptations in neurosteroids may be present in regions of brain that mediate anxiety, stress and drinking responses related to ethanol

dependence. Further studies are needed to confirm these results. The bidirectional effect across brain regions provides evidence for intracerebral regulation of neurosteroid levels. Previous studies show the peak of ethanol withdrawal at the 8 hr time point is characterized by elevated plasma corticosterone levels and increased CNS excitability, but not increased drinking. Alterations in neurosteroid levels may have functional consequences that mediate behavioral adaptations to ethanol.

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36. HDAC2-Induced Chromatin and Synaptic Remodeling in Amygdala: A Role in Anxiety and Alcoholism

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Background: Evidence indicates that both genetic and environmental risk factors play a role in the development of alcoholism. Also, innate anxiety levels have been shown to promote alcohol intake. Epigenetic mechanisms, such as histone modifications which regulate gene expression, have emerged as a promising area of research to better understand the molecular mechanisms of human diseases, including psychiatric and alcohol abuse disorders. Alcohol-preferring (P) and non-preferring (NP) rats have been selectively bred for higher and lower alcohol preference, respectively and P rats displayed anxiety-like behaviors compared to NP rats. Here, we investigated the role of histone deacetylase (HDAC) 2-induced chromatin remodeling and associated regulation of synaptic plasticity in the amygdala in anxiety and alcoholism using P and NP rats as an animal model.

Methods: Adult male P and NP rats were used in this study. In order to explore the role of specific HDAC isoforms in anxiety and alcoholism, we examined differences in the levels of HDAC2, HDAC4 and histone acetylation in the amygdaloid brain regions of P and NP rats. We also examined effects of acute ethanol (1g/kg, IP) exposure on anxiety levels and on HDAC2 and HDAC4 protein levels and histone acetylation(H3-K9) in the amygdaloid brain structures of P and NP rats. We then employed siRNA strategy to specifically knockdown HDAC2 expression in the CeA of P rats. Using behavioral paradigms, we tested the rats for anxiety-like behaviors and voluntary alcohol consumption.We analyzed the levels of HDAC2 mRNA and protein, and associated histone acetylation in amygdaloid brain regions. We also utilized chromatin immunoprecipitation (ChIP)-linked real-time PCR to explore how reduced HDAC2 affects acetylated histone H3 associated expression of synaptically active genes, such as activity-regulated cytoskeleton-associated protein (Arc) and brain-derived neurotrophic factor (BDNF). The dendritic spine density (DSD) was measured using Golgi-Cox staining.

Results: It was found that baseline amygdaloid nuclear, but not cytosolic HDAC activity was higher in P compared with NP rats. We also found that protein levels of HDAC2, but not HDAC4 were higher in the central nucleus of amygdala (CeA) and medial nucleus of amygdala (MeA), but not in basolateral amygdala (BLA) of P rats compared with NP rats. Higher HDAC2 levels were associated with decreased histone (H3-K9) acetylation in the CeA and MeA, but not in BLA of P compared with NP rats. Acute ethanol produced anxiolytic effects in P rats and decreased HDAC2 but not HDAC4 protein levels and increased histone (H3-K9) acetylation in the amygdaloid structures of P rats. Acute ethanol did not produce anxiolytic effects in NP rats and had no effect on HDAC2 levels or histone acetylation in the amygdaloid structures of NP rats. We have shown earlier that expression of Arc and BDNF, as well as DSD were innately lower in the CeA and MeA, but not BLA of P rats compared with NP rats. Interestingly, decreasing HDAC2 expression (mRNA and protein levels) in the CeA by HDAC2-siRNA but not by control-siRNA infusion produced anxiolytic effects and corrected deficits in histone (H3-K9) acetylation, DSD and protein levels of BDNF and Arc in the CeA of P rats. It was also found that decreased expression of HDAC2 by siRNA was able to increase acetylated histone H3-associated gene expression, such as Arc and BDNF exon 4, but not BDNF exon 1 in the amygdala of P rats. HDAC2 siRNA infusion into CeA also reduced the voluntary ethanol intake of P rats as measured by the two-bottle free choice paradigm.

Discussion: These results indicate that upregulation of HDAC2 in the neurocircuitry of the amygdala may be responsible for the condensed chromatin architecture and decreased expression of Arc and BDNF, thereby decreasing dendritic spines in the CeA of P rats compared with NP rats. These epigenetic modifications may be involved in the comorbidity of anxiety and alcoholism (Supported by NIH-NIAAA grants and VA Merit and Career Scientist Grants to SCP).

Disclosure: S. Pandey: None. **S. Moonat:** None. **H. Zhang:** None. **A. Sakharkar:** None.

37. Characteristic of Plasticity Facilitated by Intravenous Neural Stem Cell Transplantation in FASD Model

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Background: Fetal period alcohol exposure is suggested to be seriously influenced on the regulation of brain neural network formation. Since it is well known that the abnormal brain development can induce behavioral problems including cognition, attention and social function, the promotion of maintenance and the repair of inadequate neural network is a key strategy for psychiatric disease treatment. In the previous study, we have demonstrated the usefulness of intravenous transplantation of neural stem cells (NSCs) to fetal alcohol spectrum disorder (FASD) model rats for the purpose of reconstructing the impaired neural network and investigating the possibility of regenerative therapy for patients with neurobehavioral deficits of FASD. We have shown the potential migration of transplanted NSCs into the brain by visualizing a fluorescent cell marker and radioisotope, as well as the potential recovery of behavioral abnormalities observed in FASD model rats including the impairment of anxiety-like behavior, memory/cognitive function, and social interaction.

Methods: In the present study, to investigate the neurobiological mechanism of behavioral change by NSC transplantation, we assessed the characteristics of transplanted cells and interneurogenesis function in some brain field.

Results: In the immunohistochemical analysis, it was revealed that the GABAergic and/or Cholinergic interneurons were increased in amygdale, DG, cingulated cortex and putamen areas in the model rat. In addition, in the amygdala and cingulate cortex, intravenous NSC transplantation appeared to regenerate the expression of post-synaptic density protein 95 (PSD95) which was decreased in FASD model rats.

Discussion: These results indicate that intravenous NSC transplantation might have the potential to become a new therapeutic approach for treatment of FASD patients, by their effect of neural network repair activity, through the plasticity change of neurogenesis and synapse formation.

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38. Peripheral Markers of Psychostimulant Abuse
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Background: Long term administration of, or addiction to psychostimulants results in neuroadaptive changes at the molecular, cellular, anatomical, physiological, biochemical and behavioral levels. Detected in preclinical or clinical research, these findings form the basis of designating addiction as a brain disease. Notwithstanding this accumulating evidence, no consensus has emerged in detecting a biological marker(s) of addiction in readily accessible peripheral fluids (e.g. blood or urine). In pursuit of this goal, we investigated whether chronic exposure to psychostimulants (methamphetamine, MDMA or ecstasy, cocaine) in mice and secondarily in primates leave a biological trace of long term exposure. We administered methamphetamine or MDMA in various dosing regimens to mice and determined drug effects on expression of genes encoding axonal guidance molecules in blood. We also examine whether long term self-administration of cocaine by squirrel monkeys results in neuroadaptive changes in kidney, which conceivably would be reflected by kidney cells secreted in urine samples.
Methods: Mice (adolescent or adult) were treated repeatedly with various doses of methamphetamine or MDMA for 6-10 days and blood samples removed. RT-PCR was conducted on the samples and changes in gene expression were calculated. Squirrel monkeys were permitted to self-administer cocaine for various periods of time in the course of another study. Euthanasia was followed by removal of kidneys and RT-PCR was conducted on kidneys
Results: Significant changes in gene expression of axonal guidance molecules were detected in blood samples and in kidney tissues, were found in animals treated with psychostimulatn drug of abuse compared with saline controls.
Discussion: Our study provides preliminary evidence that psychostimulant drugs of abuse produce adaptive changes in genes expressed in blood and kidney cells. These findings conceivably may provide an accessible source of fluids to detect exposure and length of exposure to addictive drugs.
Disclosure: B. Madras: Part 1: PREXA Pharmaceuticals Alseres. **B. Constant:** None. **T. Walsh:** None. **L. Ogawa:** None. **G. Miller:** None. **E. Vallender:** None. **S. Westmoreland:** None.

39. HMGB1 and TLR increase Neuroinflammation and Neurodegeneration in Alcoholic Brain
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Background: Neuroinflammation is associated with depression, alcoholism and other brain disease. High-mobility group box 1 (HMGB1) is an agonist at receptors for damage or danger associated molecular patterns (DAMPs) that include the Toll-Like Receptors (TLR), a family of at least 13 mammalian receptor genes, that increase neuroimmune gene expression in brain through activation of NFkB transcription. HMGB1 is a nuclear protein expressed in most cells and released by multiple stimuli. DAMPS and TLRs have low expression in controls, but are increased in most brain diseases. Neuroimmune activation in brain persists for long periods of time beyond that found for systemic neuroimmune activation. HMGB1-TLR signaling may regulate brain neuroimmune and neuropathological responses.
Methods: Human, mouse and rat brain are investigated using immunohistochemistry (IHC), western protein analysis and RTPCR (mRNA). Hippocampal-entorhinal cortex (HEC) cultures were used with small interfering mRNA and drugs to follow signaling, mice were treated with ethanol (C57Bl/6, 5 gm/kg/day-10 days) to follow ethanol induced changes in brain in vivo and post-mortem human alcoholic brain to confirm humans show changes similar to mouse brain and HEC cultures.
Results: Post-mortem human alcoholic orbital frontal cortex, chronic ethanol treated mouse orbital frontal cortex (C57Bl/6) and rat HEC cultures treated with ethanol show increased levels of HMGB1 and TLR2, TLR3 and TLR4 expression. HEC culture studies find ethanol increases HMGB1 expression (mRNA and protein) and releases HMGB1 into the media. HEC and mouse studies find ethanol upregulation of HMGB1 and DAMPS is associated with increases in NFkB transcription of proinflammatory innate immune genes including TNFα, IL1β, IL6 and MCP1. In HEC, HMGB1-TLR antagonists (e.g. siRNA, naltrexone, LPS-Ra) block ethanol induction of proinflammatory innate immune genes and inhibition of neurogenesis. In mice, ethanol induction of HMGB1 and TLR modestly increase proinflammatory gene induction, but markedly potentiates proinflammatory gene induction by systemic TLR agonists LPS (TLR4) and PolyIC (TLR3). NADPHoxidase, a superoxide forming enzyme, is an innate immune gene increased by TLR agonists, ethanol alone and combinations of ethanol-TLR treatments. Increased expression of NADPHoxidase corresponds with increased formation of reactive oxygen species and markers of neuronal death (activated caspase-3 and fluoro-Jade B) in cortex. Human post-mortem alcoholic brain also shows elevated NADPHoxidase using IHC. Antagonists of microglial activation (minocycline) and TLR4 receptors (naltrexone) as well as inhibition of NADPHoxidase (DPI) protect mouse brain from ethanol-TLR agonist increased neuroinflammation and neurodegeneration.
Discussion: Ethanol increases expression of HMGB1, TLR and NADPHoxidase. Ethanol upregulation of these genes potentiates TLR3 and TLR4 responses in brain. Ethanol released HMGB1 acts on DAMP receptors, particularly TLR4, to increase neuroinflammatory gene expression. NADPHoxidase induction increases oxidative stress and neuronal death. Blockade of microglial activation, TLR receptors or NADPHoxidase protects against neuropathology. These studies indicate that HMGB1-DAMP signaling contribute to alcoholic brain disease and possibly other brain pathology. (Supported by NIAAA).
Disclosure: F. Crews: Part 1: none, Part 5: School of Medicine University of North Carolina. **L. Qin:** None. **J. Zou:** None.

40. A Double-Blind, Placebo-Controlled Trial of Topiramate for Pathological Gambling
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Background: Pathological gambling (PG) is an impulse control disorder characterized by recurrent gambling thoughts and behaviours that impair social functioning. Earlier studies suggested that topiramate may be effective in treating some impulse control disorders. We conducted the first randomized, controlled trial of topiramate in PG.
Methods: PG patients were randomized to topiramate (N = 20) or placebo (N = 22) in this 14-week, double-blind, placebo-controlled, parallel-group trial. The primary outcome measure was change in the obsessions subscale of the Yale-Brown Obsessive-Compulsive Scale Modified for Pathological Gambling.
Results: Mixed regression models (time [weeks] $\times$ treatment) revealed no significant treatment effect of topiramate on the primary or secondary outcome measures. The most statistically robust findings involved reducing the Barratt Impulsiveness Scale

(BIS) total score and Motor and Non-Planning subscale scores, for which topiramate outperformed placebo at merely a trend level ($p < 0.1$).

Discussion: The observed trend in BIS score reductions may warrant further investigation to study whether topiramate reduces clinically important impulsivity in PG. Treatment studies with larger samples and less stringent exclusion criteria are needed to produce results that can be generalized to pathological gamblers in the community

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41. Voltage Dependent Behavioral or Emotional Induction Associated with Lack of Improvement in Subsyndromal Mood Ratings following Subthalamic Nucleus Deep Brain Stimulation for Parkinson's Disease

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Background: Deep Brain Stimulation (DBS) is FDA approved for treatment-refractory Parkinson's disease (PD). Despite significant motoric improvement, complex emotional and behavioral changes experienced intraoperatively or while optimizing stimulation parameters have been reported both in PD (Krack *et al*., 2001, Chopra *et al*., 2011) and treatment-refractory depression patients (Mayberg *et al*., 2005). This study assessed the prevalence and outcome of behavioral or emotional induction (EBI) spontaneously reported while optimizing stimulator parameters in PD patients.

Methods: 54 consecutive PD patients were enrolled in an IRB-approved, 6-month follow up study. All subjects were bilaterally implanted for STN DBS. Patients' spontaneous utterances or behaviors such as feeling "at ease", "weird like punch drunk", "spacey", "messed up", or "crying" were recorded during testing stimulator thresholds or DBS parameter optimization. Stimulation parameters (voltage, pulse width, and frequency) were recorded at each of the 8 contacts (4 for each electrode). Motor symptoms were assessed by using United Parkinson's Disease Rating Scale (UPDRS). Changes in mood were assessed at baseline and each visit using Beck Depression Inventory (BDI), Hamilton Depression scale 17-item (HAM-D), and Young Mania Rating Scale (YMRS). Levodopa equivalent daily dosages (LEDD) were reported at each visit. Two-tailed Student t-test was used to compare the change in mood rating scores (BDI, HAM-D, and YMRS), and LEDD from baseline to end visit between the EBI (+) and the EBI (-) groups.

Results: 61% (n = 33) of PD subjects had a clinical comorbid psychiatric disorders [35% (n = 19) with depression, and 26% (n = 14) with bipolar spectrum or impulse control/ mania secondary to PD medications]. There was an overall improvement in motor symptoms (UPDRS) from off to on stimulation at baseline (35.2 ± 10.06 to 16.40 ± 7.91), and LEDD dose from baseline to end visit (from 1636.19 ± 1020.63 to 1065.23 ± 823.25), and mood symptoms from baseline to end visit: (HAM-D: from 8.69 ± 4.21 to 5.74 ± 4.79, BDI: from 9.02 ± 7.66 To 6.40 ± 6.09, and YMRS: from 2.02 ± 2.57 to 1.24 ± 1.57).

EBI was identified in 15 patients (depression: n = 5, BP/ impulse control disorder: n = 2, No comorbid psychiatric condition n = 8) and 29 episodes (some patients reported EBI during more than one visit) (relax = 4, euphoria = 4, crying = 4, anxiety = 13, cognitive deficits = 4)

There was an association between mean stimulation voltage (R/L) and the change in EBI from relaxed to euphoria, then crying, and anxiety and finally feeling cognitively impaired. Mean voltage (± SD) for relaxed (n = 4, R: 1.75 ± 0.35, L: 1.33 ± 0.25), euphoria (n = 4, R: 2.0 ± 0, L: 2.57 ± 0.12), crying (n = 4, R: 2.5 ± 0, L: 2.3 ± 0.98), anxiety (n = 13, R: 3.15 ± 0.64, L: 2.47 ± 0.94), and cognitive deficits (n = 4, R: 3.2 ± 0.66, L: 4.0 ± 0).

Compared to EBI (-) (n = 28), EBI (+) group (n = 14)* showed significantly less improvement in depressive symptoms as measured by change in BDI (Δ BDI) from baseline to end visit (Table 1)

	EBI (+)		EBI (−)		t-test
Change from baseline to end visit (Δ)	**N**	**Mean ± SD**	**N**	**Mean ± SD**	**p-value***
BDI	14	0.5 ± 6.1	28	−3.8 ± 6.4	0.045
YMRS	14	−0.4 ± 2.1	28	−1.0 ± 2.4	0.378
HAM-D	14	0.0 ± 7.2	28	−3.8 ± 4.4	0.044
QLES	14	0.1 ± 0.9	29	0.5 ± 0.9	0.251
LEDD	14	−454.8 ± 505.7	32	−600.6 ± 505.7	0.494

*One subject withdrew from the study

Discussion: A wide range of emotions and behaviors were abruptly induced secondary to STN stimulation in PD patients. These reported emotions seem to spread from a sense of relaxation to euphoria progressing to crying and anxiety and finally feeling cognitively impaired. This unique pattern seems to coincide with a corresponding increase in voltage at either stimulating electrodes. Interestingly, the patients who experienced these acute changes in behavior did not show a significant improvement in depressive symptoms compared to the group of patients who did not experience emotional or behavioral induction. Further research is needed to understand the anatomical and neurotransmitter basis for these intriguing findings.

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42. Predictive Factors of Efficacy of Adjunct Extended Release Quetiapine Fumarate (Quetiapine XR) in Patients with Major Depressive Disorder

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Background: Although a number of pharmacotherapies are available for the treatment of major depressive disorder (MDD), the majority of patients (50-60%) do not experience an adequate response to initial treatment. Understanding of individual patient characteristics that may moderate their treatment response is limited. Therefore, identification of potential predictors of response to different treatment strategies would help optimize outcomes. Two studies have evaluated extended release quetiapine fumarate (quetiapine XR) as adjunct to ongoing antidepressant therapy in patients with MDD and an inadequate response to prior antidepressant treatment (D1448C00006 [El-Khalili *et al. Int J Neuropsychopharmacol* 2010; 13: 917-932] and D1448C00007 [Bauer *et al. J Clin Psychiatry* 2009; 70: 540-549]). In both studies, adjunct

quetiapine XR 300 mg/day significantly reduced mean Montgomery-Åsberg Depression Rating Scale (MADRS) total score from randomization to Week 6 compared with placebo + antidepressant; adjunct quetiapine XR 150 mg/day significantly improved MADRS total score in one study. These post-hoc analyses of pooled data from the two adjunct therapy studies of quetiapine XR examined clinical and demographic characteristics that may be potential predictors of response.

Methods: Pooled data were analyzed from the two previously published, similar, 6-week, double-blind, placebo-controlled adjunct therapy studies (D1448C00006 and D1448C00007) of quetiapine XR (150 or 300 mg/day) in patients with MDD and an inadequate response to antidepressant. All analyses were performed on the pooled modified intent-to-treat (MITT) population (n = 616 quetiapine XR [both doses pooled]; n = 303 placebo). The effects of psychiatric history and baseline demographic and disease characteristics on efficacy were evaluated in two patient subgroups based on percentage reduction in MADRS total score at Week 6: 50% reduction (responders: n = 345 quetiapine XR, n = 140 placebo) versus <50% (non-responders: n = 271 quetiapine XR, n = 163 placebo); 75% reduction (responders: n = 175 quetiapine XR, n = 60 placebo) versus <25% (non-responders: n = 125 quetiapine XR, n = 89 placebo).

A further analysis evaluated the impact of baseline Clinical Global Impressions – Severity (CGI-S) score and number of episodes (0, 1, 2-3, 4-10, 10) in both the previous year and lifetime on MADRS total score change at Week 6. The effect of baseline MADRS individual item scores (items 1-10) on change in Clinical Global Impressions – Improvement (CGI-I) score at Week 6 was also evaluated.

All data were last observation carried forward (LOCF) and descriptive statistics were provided for these analyses.

Results: The comparison between responders and non-responders (defined by <50%/50% or <25%/75% reduction on MADRS total score) with regard to psychiatric history and baseline demographic and disease characteristics did not reveal any definitive predictive factors based on the criteria used. In addition, the analysis of efficacy outcomes by baseline parameters (CGI-S score, number of depressive episodes, and MADRS item scores) did not reveal any definitive predictive factors.

Discussion: In this pooled analysis of adjunct quetiapine XR (150 and 300 mg/day) in patients with MDD and an inadequate response to prior antidepressant treatment, no major differences between responders and non-responders were observed overall with regard to psychiatric history and baseline patient demographic and disease characteristics. In addition, there was no suggestion of a predictive association between the parameters assessed (baseline CGI-S score, number of depressive episodes, and baseline MADRS item scores) and efficacy outcomes for adjunct quetiapine XR. Further investigation may be required to fully understand any predictive factors for response with adjunct quetiapine XR in MDD; additional analyses including logistic regression analysis may be useful in this.

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43. A Randomized Trial evaluated the Safety and Efficacy of Levomilnacipran SR in the Treatment of Major Depressive Disorder

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Background: Levomilnacipran (1*S*, 2*R*-milnacipran) is a potent and selective norepinephrine and serotonin reuptake inhibitor (SNRI) in clinical development for the treatment of major depressive disorder (MDD). In vitro studies of levomilnacipran demonstrated potent dual reuptake inhibition that was selective and specific, with preference for the NE transporter. The sustained-release (SR) formulation was developed to allow once-daily dosing. This Phase III study evaluated the efficacy, safety, and tolerability of levomilnacipran SR fixed doses compared with placebo in adult patients with MDD.

Methods: Protocol LVM-MD-01 (NCT00969709) was a double-blind, multicenter, randomized, placebo-controlled, parallel-group, fixed-dose study. Community-dwelling patients from US clinics, ages 18-65 years, who met DSM-IV-TR criteria for MDD with an ongoing major depressive episode 8 weeks duration, a score 30 on the Montgomery-Asberg Depression Rating Scale-Clinician-Rated (MADRS-CR), and a score 26 on MADRS-Self-Rated were enrolled. After a 1-week placebo lead-in, patients were randomized (1:1:1:1) to receive placebo or levomilnacipran 40, 80, or 120 mg once daily for 8 weeks of double-blind treatment; a 2-week double-blind down-taper followed. Patients and all study staff were blinded to group assignment until database was locked, using a computer-generated schedule and coded medication labels to conceal allocation. Levomilnacipran SR was initiated at 20 mg/d and up-titrated to the targeted dose over 7 days. The primary efficacy endpoint was change from baseline to end of Week 8 in MADRS-CR total score; the primary analysis was a mixed-effects model for repeated measures (MMRM) using the intent-to-treat (ITT) population. Sensitivity analyses using last observation carried forward (LOCF) and pattern-mixture model approaches were also conducted. Secondary efficacy endpoint, change from baseline to Week 8 in Sheehan Disability Scale (SDS) total score, was analyzed using a similar approach. Safety and tolerability were

evaluated via adverse events (AEs), clinical laboratory measurements and vital signs, ECGs, and the Columbia-Suicide Severity Rating Scale (C-SSRS). The study was funded by Forest Research Institute.
Results: Of the 713 patients who were randomized and received treatment (Safety Population: n = 176, 178, 179, and 180 for placebo, 40-, 80-, and 120-mg levomilnacipran SR, respectively), 71% completed the study (n = 138 [78%], 130 [73%], 121 [68%], and 117 [65%] for placebo, 40-, 80-, and 120-mg levomilnacipran SR, respectively). Baseline and demographic characteristics were similar among groups. The least squares mean difference (LSMD) for change from baseline in MADRS-CR total score (primary efficacy) was significantly superior to placebo in all dose groups: -3.23, ($P = 0.0186$), -3.99 ($P = 0.0038$), and -4.86 ($P = 0.0005$) for levomilnacipran SR 40, 80, and 120 mg, respectively; results remained significant after adjustment with the Hochberg procedure for multiplicity. Statistically significant differences were also seen in LOCF analyses; LSMD were -2.56 ($P = 0.0410$), -3.45 ($P = 0.0058$), and -3.43 ($P = 0.0063$) for levomilnacipran SR 40, 80, and 120 mg, respectively. AEs led to discontinuation of significantly more levomilnacipran SR than placebo patients: 2%, 7% ($P = 0.0185$), 15% ($P < .001$), and 7% ($P = 0.0316$) of placebo and levomilnacipran SR 40-, 80-, and 120-mg patients, respectively. Double-blind period treatment-emergent AEs (TEAEs) occurred in 64%, 76%, 83%, and 77% of placebo and levomilnacipran SR 40-, 80-, and 120-mg patients, respectively. TEAEs that occurred in 10% of any treatment group comprised headache, nausea, constipation, dry mouth, increased heart rate, and hyperhidrosis. Serious AEs were reported by 2 (1%) patients taking levomilnacipran SR 40 mg (chest pain and deep vein thrombosis; aggression) and 1 (0.6%) patient taking 80 mg (cytomegalovirus mononucleosis).
Discussion: Levomilnacipran SR 40, 80, and 120 mg/d demonstrated significant improvement relative to placebo in MADRS-CR. Significantly more patients in the levomilnacipran SR groups discontinued due to AEs. No other clinically meaningful differences were noted between active treatment groups versus placebo.
Disclosure: A. Bose: Part 5: Forest Research Institute. **N. Rosenthal:** None. **C. Gommoll:** Part 5: Forest Research Institute. **C. Chen:** Part 5: Forest Research Institute. **P. Ross:** Part 5: Forest Research Institute.

44. Mitochondrial Enhancement in Bipolar Disorder: A Placebo-Controlled Trial of Acetyl-L-Carnitine and Alpha-Lipoic Acid in the Treatment of Bipolar Depression

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Background: Bipolar disorder may be associated with mitochondrial dysfunction. Therefore, agents that enhance mitochondrial functioning may represent a novel therapeutic approach to bipolar disorder. Acetyl-L-carnitine (ALCAR), a naturally occurring mitochondrial metabolite, has been shown to improve mitochondrial function and increase mitochondrial energy production in both animal and human studies, and has demonstrated efficacy in a variety of depressive spectrum disorders making it an intriguing candidate treatment for the depressed phase of bipolar disorder. However, while ALCAR may increase energy production, it may also cause an increase in the production of reactive oxygen species. Alpha-lipoic acid (ALA), a mitochondrial coenzyme, is a potent antioxidant, and thus an ideal agent to use in combination with ALCAR to increase mitochondrial metabolic activity without a concomitant increase in oxidative stress.Therefore, we performed a randomized placebo-controlled trial of ALCAR plus ALA in the treatment of bipolar depression, and assessed markers of cerebral energy metabolism using phosphorus magnetic resonance spectroscopy (^{31}P-MRS). We hypothesized that treatment with ALCAR/ALA would have significantly greater efficacy than placebo, and that ALCAR/ALA treatment, but not placebo, at both week 1 and week 12 would be associated with an increase relative to baseline in cerebral concentrations of the high-energy phosphate compounds phosphocreatine (PCr) and beta-nucleoside triphosphate (bNTP) and an increase in intracellular pH.
Methods: We administered ALCAR (1000-3000 mg/daily) plus ALA (600-1800 mg/daily) or matching placebo for 12 weeks to 40 patients with bipolar depression. Our primary clinical outcome measure was the Montgomery-Asberg Depression Rating Scale (MADRS), and secondary clinical outcome measures were the Hamilton Depression Rating Scale (HAM-D), Clinical Global Impression Scale for Severity (CGI-S), and the Young Mania Rating Scale (YMRS). Statistical analysis used longitudinal random effects models. We obtained imaging data at baseline, week 1, and week 12 of treatment in 20 patients using phosphorus three-dimensional chemical-shift imaging (^{31}P 3D-CSI) at 4 Tesla. Our primary imaging region of interest was the anterior cingulate cortex (ACC). Exploratory regions of interest included the parieto-occipital cortex (POC), frontal cortex, thalamus, and whole brain.
Results: We found no significant difference between ALCAR/ALA and placebo on change from baseline in the MADRS in both the longitudinal (mean difference [95% confidence interval]: -1.4 [-6.2, 3.4], $p = 0.58$) and last-observation-carried-forward (-3.2 [-7.2, 0.9], $p = 0.12$) analyses. The mean (SD) study doses of ALCAR and ALA at endpoint were 2275 (751) mg daily and 1365 (450) mg daily, respectively. We found virtually no evidence for a difference at week 1 or week 12 between groups in ^{31}P-MRS markers of cerebral energy metabolism. We found no significant association between change in any of our primary metabolites of interest and change in MADRS in any of the regions examined. Reduction in whole brain total NTP (alpha-, beta- and gamma-NTP) levels from baseline to week 1 was associated with reduction in MADRS scores ($p = .02$) in patients treated with ALCAR/ALA. However, this was likely a chance finding attributable to multiple statistical comparisons.
Discussion: Overall, our data suggest that treatment with ALCAR/ALA at the dose and duration used in this study does not have antidepressant effects in depressed bipolar patients and does not significantly enhance mitochondrial functioning in this patient group. Further investigation into the specific molecular underpinnings of mitochondrial dysfunction in bipolar disorder is needed to ultimately conceptualize and develop potential new treatments that potently enhance mitochondrial functioning through well-understood targeted mechanisms.
Disclosure: B. Brennan: Part 1: Eli Lilly, Transcept Pharmaceuticals,, Part 4: Eli Lilly, Transcept Pharmaceuticals. **J. Jensen:** None. **J. Hudson:** Part 1: Alkermes, Eli Lilly, Ortho-McNeil Janssen Scientific Affairs, Otsuka, Pfizer, Roche, Shire, Part 2: Pfizer, Part 3: None, Part 4: Alkermes, Eli Lilly, Ortho-McNeil Janssen Scientific Affairs, Otsuka, Shire, Part 5: No. **C. Coit:** None. **A. Beaulieu:** None. **H. Pope, Jr.:** Part 1: Alkermes. **P. Renshaw:** Part 1: Kyowa Hakko Kirin, Ridge Diagnostics. **B. Cohen:** None.

45. Adjunctive Aripiprazole Response Rates in Major Depressive Disorder Patients who Exhibit No or Minimal Improvement on Antidepressant Monotherapy

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Background: The 2010 American Psychiatric Association (APA) Guidelines[1]outline several treatment options, including augmenta-

tion with a second agent, for patients with major depressive disorder (MDD). Augmentation strategies have traditionally been reserved for MDD patients with at least a partial response to antidepressant therapy (ADT).[2] However, contrary to that line of thinking, recent analyses have shown that the benefits of adjunctive aripiprazole are not limited to ADT partial responders but extend to ADT minimal responders, as well.[3,4] Moreover, these benefits occurred as early as Week 1. Given these findings, a clinical question that remains is whether adjunctive aripiprazole would produce significant and rapid benefits in patients with no discernable improvement with ADT. To address this important question, we used the clinically meaningful, single-item Clinical Global Impression – Improvement (CGI-I) score to characterize MDD patients as either ADT monotherapy Minimal Improvers or ADT monotherapy Non-Improvers. Since clinicians may not consider using adjunctive aripiprazole in patients with no ADT response, this *post-hoc* analysis was conducted to assess clinically meaningful improvement (response rates) after the addition of adjunctive aripiprazole or placebo in ADT Minimal Improvers and also ADT Non-Improvers.

Methods: Data from three nearly identical studies of aripiprazole augmentation of ADT were pooled. Eligible patients were aged 18–65 years and had a major depressive episode (Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition –Text Revision [DSM-IV-TR] criteria) lasting 8 weeks and a 17-item Hamilton Rating Scale for Depression [HAM-D17] total score 18. Patients had to have an inadequate response to one to three adequate ADT trials of 6 weeks duration (3 weeks for combination treatments). These patients were then enrolled in a study with an 8-week prospective ADT phase followed by a 6-week randomized, double-blind phase that evaluated the efficacy and safety of adjunctive aripiprazole in patients with an inadequate response ($<$50% reduction in HAM-D17 total score, HAM-D17 total score 14, and CGI-I score 3) to ADT. Aripiprazole dosing was started at 5 mg/day and could be increased by up to 5 mg/day/week, to a maximum of 20 mg/day. This *post-hoc* analysis included the subset of randomized patients rated as Minimal Improvers (CGI-I = 3) or Non-Improvers (CGI-I = 4) at Weeks 6 and 8 of the prospective ADT monotherapy treatment period. Response rates (50% reduction in Montgomery–Åsberg Depression Rating Scale [MADRS] from the end of the prospective ADT monotherapy phase) at the end of the 6-week double-blind adjunctive treatment phase were evaluated in this subpopulation using Fisher's exact test. Number needed to treat (NNT) for response rates was also calculated.

Results: 452 Minimal Improvers (238 aripiprazole; 214 placebo) and 262 Non-Improvers (126 aripiprazole; 136 placebo) were randomized. Depressive symptoms evaluated by MADRS were significantly reduced after the addition of aripiprazole adjunctive compared to continued ADT monotherapy in both improver groups. In Minimal Improvers, a separation on MADRS total score between adjunctive aripiprazole and placebo was seen as early as Week 1 and in Non-Improvers at Week 2, and improvement was sustained to endpoint in both groups. A significantly greater proportion of patients in both groups responded to aripiprazole compared with placebo at endpoint ($p<0.01$). Importantly, significant differences between aripiprazole and placebo responders were observed and sustained to endpoint as early as Week 1 among Minimal Improvers and Week 4 among Non-Improvers ($p<0.01$ each). The NNT to achieve a response for Minimal Improvers was nine (95% confidence interval [CI]: 4.8, 27.7) and for Non-Improvers was eight (95% CI: 4.4, 21.5). The total daily dose of aripiprazole was similar between groups. The rates of adverse events for Minimal Improvers and Non-Improvers were also similar.

Discussion: The results support the efficacy of adjunctive aripiprazole in patients with both minimal and no improvement on ADT monotherapy, and the NNTs for response indicate that the benefits are clinically meaningful. Using a starting dose of 5 mg/day, efficacy of adjunctive aripiprazole was seen as early as Week 2 in subjects who did not improve at all on ADT monotherapy; this rapid onset is helpful to both clinicians and patients, and allows important treatment decisions to be made early in the course of therapy.

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Disclosure: D. Casey: Part 1: Daniel E. Casey has served as a consultant for Abbott Laboratories, Bristol-Myers Squibb, Dainippon Sumitomo Pharmaceuticals, Genentech, Janssen Pharmaceuticals, Merck, NuPathe Inc., Pfizer Inc., Solvay Pharmaceuticals and Wyeth Pharmaceuticals. He has served on the speakers' bureau for Abbott Laboratories, Bristol-Myers Squibb, Janssen Pharmaceuticals, Merck and Pfizer Inc. **K. Laubmeier:** Part 5: Bristol-Myers Squibb employee. **E. Bellocchio:** Part 1: Owns Bristol-Myers Squibb stock., Part 5: Bristol-Myers Squibb employee. **J. Eudicone:** Part 5: Bristol-Myers Squibb employee. **R. McQuade:** Part 5: Employee of Otsuka Pharmaceutical Development and Commercialization. **R. Baker:** Part 5: Employee of Otsuka Pharmaceutical Development and Commercialization. **Z. Rahman:** Part 5: Employee of Bristol-Myers Squibb since September 2010. Prior to that Zia Rahman was an employee of Pfizer Inc.

46. Reliability and Validity of the Fatigue Associated with Depression (FAsD) Questionnaire in a Clinical Trial of Patients with Major Depressive Disorder who Partially Respond to SSRI Treatment

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Background: Fatigue is one of the most frequent and bothersome symptoms to occur in patients with major depressive disorder (MDD). Fatigue is associated with lower psychosocial functioning and is highly prevalent among patients who partially respond to antidepressant treatment. The Fatigue Associated with Depression (FAsD) questionnaire was developed to assess the experience and impact of fatigue specifically among patients with MDD. The FAsD is a 13-item instrument assessing 2 domains [Fatigue Experience (items 1-6) and Fatigue Impact (items 7-13)] and each item is scored on a scale from 1 to 5, with higher scores indicating more frequent/severe fatigue. Domain scores are reported as a mean of domain items, and a total mean score is computed. Previous validation of the FAsD has been established in a large depressed outpatient clinical sample. The purpose of this analysis was to validate the FAsD using data from a pharmaceutical clinical trial in patients with MDD who partially responded to selective serotonin reuptake inhibitor (SSRI) treatment (including citalopram, escitalopram, fluoxetine, paroxetine, and sertraline).

Methods: Data for this analysis was from a 10-week acute, randomized, double-blind, placebo-controlled trial with adjunctive flexibly-dosed LY2216684 (a potent and selective norepinephrine reuptake inhibitor) 6–18 mg once daily or placebo. Key inclusion criteria were SSRI treatment for 6 weeks, partial response by investigator's opinion, and GRID 17-item Hamilton Depression Rating Scale total score 16. Efficacy and safety results from this trial have been presented elsewhere. Psychometric analyses were conducted using data from week 0 before administration of any study medications. Known-groups validity was conducted using data from week 10. Internal consistency reliability was assessed for the FAsD Experience and Impact subscales as well as the Summary Score using Cronbach's formula for coefficient alpha, while

construct validity was assessed using Spearman correlations. All analyses were performed on the pooled sample of patients.
Results: Of 243 patients included in the analysis at week 0, 68.3% were female, mean age was 45 years, and the mean (SD) depression score was 30.77 (5.55) on the Montgomery-Asberg Depression Rating Scale. Mean (SD) FAsD scores were 3.88 (0.78) for the Experience Subscale, 3.62 (0.89) for the Impact Subscale, and 3.75 (0.76) for the Summary Score. The subscales and summary score of the FAsD demonstrated strong internal consistency reliability (Cronbach's alphas: 0.86-0.92). The FAsD demonstrated construct validity through strong correlations with measures assessing functioning (measured by the Sheehan Disability Scale) and fatigue/energy (measured by the Visual Analog Scale-Fatigue and Massachusetts General Hospital Cognitive and Physical Functioning Questionnaire), in contrast to relatively low correlations with instruments assessing anxiety (measured by the Hospital Anxiety Depression Scale). The FAsD also demonstrated known-groups validity by discriminating among groups of patients categorized based on clinician ratings on the global severity of depression measure (CGI-S).
Discussion: In the current analysis, the FAsD questionnaire demonstrated internal consistency reliability, convergent, divergent, and known-groups validity when used in a clinical trial of patients who partially respond to SSRI treatment. Similar to prior psychometric evaluations of the FAsD, results from this analysis suggest that the instrument provides a reliable and valid patient-reported assessment of depression-related fatigue in patients with MDD. Future psychometric analyses on the FAsD questionnaire should consider establishing a responder definition and the sensitivity to change within a clinical trial sample of patients with MDD.
Disclosure: P. Classi: Part 1: Peter is an employee and shareholder of Eli Lilly and Company., Part 2: Peter is an employee and shareholder of Eli Lilly and Company., Part 3: Peter is an employee and shareholder of Eli Lilly and Company., Part 4: None, Part 5: Eli Lilly and Company. **D. Milton:** Part 1: Denai is an employee and shareholder of Eli Lilly and Company., Part 2: Denai is an employee and shareholder of Eli Lilly and Company., Part 3: Denai is an employee and shareholder of Eli Lilly and Company., Part 4: None, Part 5: Eli Lilly and Company. **J. Witkin:** Part 1: Jeffrey is an employee and shareholder of Eli Lilly and Company., Part 2: Jeffrey is an employee and shareholder of Eli Lilly and Company., Part 3: Jeffrey is an employee and shareholder of Eli Lilly and Company., Part 4: None, Part 5: Jeffrey is an employee and shareholder of Eli Lilly and Company. **R. Shelton:** Part 1: Bristol-Myers Squibb, Eli Lilly and Company, Cyberonics, Inc., Euthymics Bioscience, Evotec AG, Forest Pharmaceuticals, Gideon Richter PLC, Janssen Pharmaceutica, Medronic, Inc., Novartis Pharmaceuticals, Otsuka Pharmaceuticals, Pamlab, Inc., Pfizer, Inc., Repligen, Corp., St. Jude Medical, Inc., Part 2: None, Part 3: None, Part 4: Bristol-Myers Squibb, Eli Lilly and Company, Euthymics Bioscience, Forest Pharmaceuticals, Janssen Pharmaceutica, Novartis Pharmaceuticals, Otsuka Pharmaceuticals, Pamlab, Pfizer, Inc., Repligen, Corp., and St. Jude Medical, Inc. **L. Matza:** Part 1: Louis Matza is an employee of United Biosource Corporation, a company that received funding from Eli Lily and Company for time spent on this research, Part 5: United BioSource Corporation.

47. Cognitive-Behavioral Therapy in Women Discontinuing Antidepressant in Anticipation of Pregnancy

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Background: Recent large-scale studies demonstrate that new onset and recurrence of depressive episodes are common during pregnancy and the postpartum period. Although antidepressants (AD) are frequently used during pregnancy, concerns remain regarding a spectrum of adverse outcomes associated with fetal exposure to AD. To date, the efficacy of non-pharmacologic interventions for the purposes of relapse and recurrence prevention following AD discontinuation in this population has not been systemically investigated. The overarching goal of this study was to adapt a cognitive behavioral prevention of recurrence treatment (CBT-PR) for women with a history of recurrent major depressive disorder who planned to discontinue their maintenance AD treatment given plans to conceive.
Methods: Women who were planning pregnancy or recently pregnant with a history of major depression (documented clinical remission for at least six months), on current AD, and who independently decided to discontinue AD for pregnancy were enrolled in an open, non-randomized pilot study. Subjects received 12 weekly sessions of CBT-PR during the acute phase of the trial. After baseline assessment, subjects were assessed bi weekly by an independent rater using mood, quality of life, and reproductive status measures. Recurrence was determined using the mood module of the Mini-International Neuropsychiatric Interview (MINI) and depression severity was assessed using the Montgomery-Asberg Depression Rating Scale (MADRS).
Results: Twelve (N = 12) eligible subjects were enrolled in the pilot (mean age = 34 years, SD = 3.96). To date, eleven subjects have completed the acute phase. Average length of AD taper was 4.3 weeks (SD = 2.53; range 1-9 weeks); taper schedules were determined by the subject in collaboration with her psychiatrist based on what was clinically appropriate for the medication. Subjects had a mean of 4.18 of past episodes of major depression (range 1-15) and a mean of 1 failed attempt at AD discontinuation (range 0-3). Two subjects relapsed and treatment was re-initiated in the absence of a mood episode by one subject during the acute phase. The time to relapse following complete AD discontinuation for the two subjects who relapsed was 4.5 and 7 weeks. The subject who reinitiated AD treatment during the acute phase in the absence of a mood episode did so 1.5 weeks after discontinuing treatment. Mean baseline MADRS score was 9.7 for the subjects who relapsed or reinitiated AD treatment, and 4.5 for those who remained euthymic during the acute phase of the study. Subjects who relapsed/reinitiated treatment had a mean MADRS score of 15.33 at the time of relapse and subjects who remained euthymic during the acute phase of the study had a mean MADRS score of 9.13 at their last session.
Discussion: CBT-PR may be an alternative to AD for some women in order to prevent depressive relapse or recurrence either during attempts to conceive or during pregnancy. The extent to which euthymia is sustainable after treatment with CBT-PR in this population is an area of future needed study, the results of which may broaden treatment choices for women during this crucial time.
Disclosure: C. Psaros: Part 1: Consultant, United BioSource Corporation. Conduct third party review of fidelity to scoring conventions of measures of depression and anxiety completed as part of clinical trials, Part 2: Consultant, United BioSource Corporation. Conduct third party review of fidelity to scoring conventions of measures of depression and anxiety completed as part of clinical trials, Part 3: Consultant, United BioSource Corporation. Conduct third party review of fidelity to scoring conventions of measures of depression and anxiety completed as part of clinical trials. **M. Freeman:** Part 1: Advisory board Bristol Myers Squibb, Part 4: forest Lilly GSK. **S. Safren:** None. **M. Barsky:** None. **L. Cohen:** Part 1: Eli Lilly & Company (Advisory Board); Noven (Advisory Board), Part 4: Bristol-Myers Squibb; Ortho-McNeil Janssen Scientific Affairs, LLC; AstraZenneca; Pfizer; Bayer.

48. A Double-Blind, Placebo-Controlled Study of Selegiline Transdermal System (STS) in Depressed Adolescents

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Background: Despite consistent evidence for efficacy in adults, the clinical use of monoamine oxidase inhibitors (MAOIs) has declined at least partially due to safety concerns about food and drug interactions. Selegiline transdermal system (STS) was developed to overcome some of the limitations of orally administered MAOIs. STS delivers sustained blood levels of monoamine oxidase inhibitor (MAOI) directly into systemic circulation, thereby bypassing inhibition of MAO-A in the GI tract and liver and avoiding the need for a tyramine-restricted diet at the 6-mg/24 hr dose. STS has been FDA approved for acute and maintenance treatment of Major Depressive Disorder in adults. STS is not indicated in patients under the age of 18. The purpose of this study was to examine the safety, tolerability, and efficacy of selegiline transdermal system (STS) for adolescents with major depressive disorder (MDD). This study was an FDA post-marketing commitment.

Methods: This was a phase IV, multi-center, double-blind, placebo-controlled, randomized, flexible-dose safety and efficacy study of STS for adolescents (ages 12-17 years) meeting DSM-IV criteria for moderate to severe MDD without psychotic features. Eligible participants were randomized 1:1 to active treatment or matching placebo patches for a period of 12 weeks. Active treatment consisted of flexible dosing of STS 6 mg/24 hrs, 9 mg/24 hrs, or 12 mg/24 hrs based on a predefined dosing schedule. While no dietary modifications were required for patients on the 6 mg/24 hr dose, dietary modifications were required as per the STS label for patients who titrated to the 9 mg/24 hr and 12 mg/24 hr doses.

The primary efficacy outcome measure was the mean change from baseline to end of study (week 12 LOCF) in the CDRS-R total score. Secondary outcome measures included endpoint Clinical Global Impression of Severity (CGI-s), Clinical Global Impression of Change (CGI-c).

Results: Adolescents (N = 308) with moderate to severe MDD were randomized to either STS (n = 152) or placebo (n = 156). 215 (69.8%) subjects completed the study. The overall incidence of reported adverse events was 62.5% for STS patients and 57.7% for placebo-treated patients. Most commonly reported adverse events in both STS and placebo groups was application site reactions (STS = 24.3%; placebo = 21.8%), headache (STS = 17.1%; placebo = 16.7%), and nausea (STS = 7.2%; placebo = 7.7%). A total of 17 (5.5%) patients reported discontinuing due to adverse events (STS: n = 12, 7.9%; placebo: n = 5, 3.2%). Treatment groups did not differ on any laboratory parameters, vital signs, or ECG findings. No suspected hypertensive crises were seen in the trial. Patients on STS and placebo had significant reductions from baseline on their CDRS-R Total Score with mean reductions ± SD as follows: STS 21.4 ± 16.6; placebo 21.5 ± 16.5. Both groups had similar response rates (58.6% versus 59.3%) defined as CGI-C of 1 or 2 at study endpoint. There was no statistical difference between groups on primary or secondary endpoint measures.

Discussion: STS was generally safe and well-tolerated in adolescents with moderate to severe MDD, as evidenced by the overall adverse event profile and the lack of significant vital sign, ECG and laboratory findings. The adverse event profile seen in adolescent subjects is consistent with the STS prescribing information for use in adults. Although the STS group had significant reductions in depression, it did not demonstrate statistical superiority over placebo on any primary or secondary efficacy measures, perhaps because there was a large placebo response.

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Disclosure: M. DelBello: Part 1: Research involvement: Eli Lily, AstraZeneca, Pfizer, Otsuka, GSK, Johnson and Johnson, Somerset, Inflammation Foundation, Merck. Consulting: Pfizer, BMS, Eli Lilly, Schering-Plough Speaking: Merck, BMS, Part 2: BMS, Part 3: BMS-consulting and speaking, Part 4: Eli lilly, AstraZeneca, Pfizer, Otsuka, GSK, Johnson and Johnson, Somerset, Inflammation Foundation, Merck., Part 5: n/a. **T. Hochadel:** Part 1: Alexza Pharmaceuticals, CeNeRx Biopharma, Cephalon, Cognitive Research Corporation, Dey Pharma, Factor Nutrition, Helicon Therapeutics, Johnson & Johnson, Merck, Pharmavite, Sagene Pharmaceuticals, Somerset Pharmaceuticals, Sunovion, Vista Pharmaceuticals, Vivus., Part 2: Cognitive Research Corporation, Part 3: Cognitive Research Corporation, Part 5: Cognitive Research Corporation. **K. Portland:** Part 1: Dey Pharma, LP, Part 2: Dey Pharma, LP, Part 5: Dey Pharma, LP. **A. Katic:** Part 1: Abbott Laboratories, Alexza, Bristol-Myers Squibb, Cephalon, Corcept, Cyberonics, Dainippon Sumitomo, Eli Lilly, Forest, GlaxoSmithKline, Hisamitsu, Lundbeck, Merck, Novartis, Organon, Otsuka, Pfizer, Roche, sanofi-aventis, Sepracor, Shire, Somerset, Takeda, Targacept and Wyeth and served on a speaker's bureau for Novartis, Pfizer, and Shire., Part 2: Abbott Laboratories, Alexza, Bristol-Myers Squibb, Cephalon, Corcept, Cyberonics, Dainippon Sumitomo, Eli Lilly, Forest, GlaxoSmithKline, Hisamitsu, Lundbeck, Merck, Novartis, Organon, Otsuka, Pfizer, Roche, sanofi-aventis, Sepracor, Shire, Somerset, Takeda, Targacept and Wyeth and served on a speaker's bureau for Novartis, Pfizer, and Shire., Part 3: Abbott Laboratories, Alexza, Bristol-Myers Squibb, Cephalon, Corcept, Cyberonics, Dainippon Sumitomo, Eli Lilly, Forest, GlaxoSmithKline, Hisamitsu, Lundbeck, Merck, Novartis, Organon, Otsuka, Pfizer, Roche, sanofi-aventis, Sepracor, Shire, Somerset, Takeda, Targacept and Wyeth and served on a speaker's bureau for Novartis, Pfizer, and Shire. **A. Khan:** Part 1: Arif Khan, MD, has been a principal investigator for over 340 clinical trials sponsored by more than 65 pharmaceutical companies and 30 contract research organizations. Dr. Khan is not a shareholder of any of these companies, nor has he done any compensated consulting or speaking on their behalf. Dr. Khan received no financial compensation or incentive for authoring this abstract. Dr. Khan is the Medical Director for Columbia Northwest Pharmaceuticals and Rhine Pharmaceuticals and is also a shareholder in these two companies. Columbia and Rhine own intellectual property rights for potential therapies for Central Nervous System disorders and other medical conditions. , Part 2: Arif Khan, MD, has been a principal investigator for over 340 clinical trials sponsored by more than 65 pharmaceutical companies and 30 contract research organizations. Dr. Khan is not a shareholder of any of these companies, nor has he done any compensated consulting or speaking on their behalf. Dr. Khan received no financial compensation or incentive for authoring this abstract. Dr. Khan is the Medical Director for Columbia Northwest Pharmaceuticals and Rhine Pharmaceuticals and is also a shareholder in these two companies. Columbia and Rhine own intellectual property rights for potential therapies for Central Nervous System disorders and other medical conditions. , Part 3: Arif Khan, MD, has been a principal investigator for over 340 clinical trials sponsored by more than 65 pharmaceutical companies and 30 contract research organizations. Dr. Khan is not a shareholder of any of these companies, nor has he done any compensated consulting or speaking on their behalf. Dr. Khan received no financial compensation or incentive for authoring this abstract. Dr. Khan is the Medical Director for Columbia Northwest Pharmaceuticals and Rhine Pharmaceuticals and is also a shareholder in these two companies. Columbia and Rhine own intellectual property rights for potential therapies for Central Nervous System disorders and other medical conditions. , Part 4: none. **G. Emslie:** Part 1: Research: Biobehavioral Diagnostic Inc., Eli Lilly, Forest Laboratories, GlaxoSmithKline, Somerset Consultant: Biobehavioral Diagnostic Inc., Eli Lilly, Forest Laboratories, GlaxoSmithKline,

INC Research Inc., Lundbeck, Pfizer Inc., Seaside Therapeutics Inc., Shire, Wyeth. Speakers Bureau: Forest Laboratories, Part 2: none, Part 4: Research: Biobehavioral Diagnostic Inc., Eli Lilly, Forest Laboratories, GlaxoSmithKline, Somerset.

49. Factorial Clinical Trials for Hybrid (Explanatory and Pragmatic) Research Studies: Design for the "Optimizing Treatment for Complicated Grief" Study

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Background: Schwartz and Lellouch (1967) described the distinction between explanatory and pragmatic clinical trials, with explanatory trials aiming at discovery of new efficacious treatments and pragmatic trials aiming at clinical decision making in settings such as comparative effectiveness research (CER). Explanatory trials are usually designed to equalize associated contextual factors ("everything else being equal") in order to isolate the efficacy of the treatment under study; pragmatic trials are usually designed to optimize associated contextual factors with the interpretation of the treatment being delivered as a bundle that incorporates contextual effects associated with the treatment under study in naturalistic delivery settings. As an example, differential levels of clinician-patient interactions are usually considered a source of bias (attention bias) in explanatory trials, while the same difference is considered part of the naturalistic treatment "bundle" in pragmatic trials. While the explanatory and pragmatic perspectives are usually applied separately in most clinical studies, combination of the two paradigms can be valuable in studies of combination therapies using factorial designs. We illustrate such a hybrid approach in the design of our on-going study, Optimizing Treatment for Complicated Grief, a study of medication (citalopram) and Complicated Grief Treatment (CGT), a therapy designed specifically for the treatment of complicated grief (CG).
Methods: We employed a 2x2 factorial design, with treatment arms (1) Medication (citalopram) with clinical management, (2) Placebo with clinical management, (3) Medication with clinical management + CGT, and (4) Placebo with clinical management + CGT. Our first study aim compares Arms (1) and (2) to evaluate the efficacy of Medication for the treatment of CG from the explanatory perspective. Our second study aim compares Arms (3) and (4) to evaluate the efficacy of Medication in the presence of CGT, also from the explanatory perspective. Our third study aim compares Arms (3) and (1) to evaluate the effectiveness of CGT in the presence of Medication, from the pragmatic perspective.
Results: With the 2x2 factorial design, we chose to interpret the medication aims (aims 1 and 2) as explanatory, due to the lack of established evidence for the efficacy of medication for the treatment of CG; and the CGT aim (aim 3) as pragmatic, considering existing evidence for the efficacy of CGT for the treatment of CG, e.g., in Shear *et al*. (2005). These interpretations led to important design decisions. First, triple-blinded, placebo controlled comparisons are used for the medication aims, with the medication assignment (citalopram vs. placebo) blinded to patients, clinicians, and independent evaluators (IE's) responsible for assessments; at the same time, comparisons for the CGT aim are open-labeled to patients and clinicians, but blinded to IE's to maintain the objectivity of the assessments, with attention "bias" considered part of the treatment bundle for the naturalistic delivery of CGT under the pragmatic paradigm. Second, we employ parallel but distinct assessment schedules appropriate for each aim, with a fixed-time schedule (irrespective of treatment completion) for the explanatory aims, and a variable time schedule for the pragmatic aim. The study was launched successfully according to this hybrid design in March 2010. As of August 2011, the study has enrolled and randomized 135 patients across four sites, amounting to approximately one third of the total recruitment goal.
Discussion: Factorial clinical trials can be used to study multiple research questions simultaneously in the same study, to expedite the production of knowledge efficiently. When the research questions being studied include both explanatory and pragmatic tasks, the hybrid design such as the one use in the Optimizing Treatment for Complicated Grief study can be utilized to accommodate both sets of objectives. It is important in such hybrid studies to accommodate unique design needs for various components of the overall study.
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50. A Double-Blind, Randomized, Placebo-Controlled Long-Term Study of Aripiprazole in Children with Bipolar Disorder

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Background: Aripiprazole (APZ) is an atypical antipsychotic indicated by the United States Food and Drug Administration for the acute treatment of manic or mixed episodes associated with bipolar I disorder as monotherapy and adjunctive to lithium or valproate in pediatric patients ages 10-17. Owing to the severity and chronicity of pediatric bipolar disorder in children younger than 10 years of age, safe and effective long-term interventions are needed for that patient population. This study tested the long-term efficacy of APZ compared to placebo in children under age 10 suffering from a bipolar disorder.
Methods: Medically healthy outpatients, ages 4-9 years, meeting DSM-IV criteria for a bipolar disorder were eligible to receive up to 16 weeks of open-label treatment with APZ (Phase I) (Findling *et al*., in press). Patients ended participation in Phase I and were randomized into the double-blind phase of the study, Phase II, once they met *a priori* response criteria (treatment with APZ for a minimum of 6 weeks and 3 of 4 consecutive weeks with: Children's Depression Rating Scale-Revised (CDRS-R) < 29; Young Mania Rating Scale (YMRS) < 10; and Children's Global Assessment Scale (CGAS) > 50). Phase II, the primary focus of this poster, was a randomized, double-blind clinical trial in which stabilized patients received either ongoing APZ treatment or placebo for up to 72 weeks. Patients either remained on their current APZ dose, or began a double-blind taper during which APZ was discontinued and replaced by placebo. The treatment groups were compared on demographics, psychiatric diagnoses, weeks enrolled in Phase II, symptom ratings at time of randomization, adverse events, weight gain, and changes in safety laboratory values. Treatment efficacy was examined using two separate Kaplan-Meier survival analyses: one used "discontinuation for any reason" as the event of interest, and the other used "discontinuation due to the development of a mood episode" to quantify risk of discontinuation. Cox regression analyses were computed to examine the effects of covariates. For all analyses, significance was set at $p < 0.05$.
Results: Thirty patients were randomized to APZ while receiving an average daily dose of 0.23 (0.07) mg/kg/day after a mean of 14.3 (2.8) weeks of open-label treatment with APZ. Also, 30 patients who were being treated with 0.22 (0.07) mg/kg/day of APZ were randomized to placebo after 14.2 (2.4) weeks of open-label treatment. The two groups did not significantly differ in the time

until stabilization and randomization in Phase I ($p = 0.88$). The APZ group did not significantly differ from the placebo group in mean weight adjusted total daily dose at randomization ($p = 0.64$). No significant differences were observed between treatment groups at baseline for any demographic, diagnostic, or symptom rating variables (all p values > 0.05). Six patients randomized to receive APZ and 0 patients randomized to placebo completed the entire 72 weeks of Phase 2. For patients randomized to continued APZ therapy, time to study discontinuation for any reason and as a result of a mood event was longer compared to those randomized to placebo (any reason: $p = 0.003$; mood event: $p = 0.005$).Regardless of randomized assignment, both APZ and placebo showed substantial rates of withdrawal from maintenance treatment over the initial 4 weeks (15/30, 50% for APZ; 27/30, 90% for placebo) suggesting a possible nocebo effect. Children treated with APZ were more likely to report both stomach ($n = 10$ (33%) vs. $n = 1$ (3%)) and musculoskeletal pain ($n = 8$ (27%) vs. 0) than those who received placebo (both $p < 0.01$). There was a significant difference in weight gain from time of randomization between patients who received APZ (mean = 2.61 kg, S.D. = 3.88 kg) versus those that received placebo (mean = 0.42 kg, S.D. = 1.26 kg; $p = 0.006$). There was a significant time × treatment interaction in prolactin levels ($F = 19.76$, $df = 1, 56$, $p < 0.001$). Compared to the randomization time point, prolactin levels decreased at EOS in the APZ group, while prolactin levels at EOS in the placebo group increased.

Discussion: Results from this double-blind, placebo-controlled maintenance trial suggest that APZ may be beneficial in the long-term treatment of pediatric patients with a bipolar disorder following stabilization with open-label APZ. Considering the low completion rate, further treatment research is needed in order improve long term outcomes in this population. **Reference:** Findling RL, McNamara NK, Youngstrom EA, *et al*. An open-label study of aripiprazole in children with a bipolar disorder. J Child Adolesc Psychopharmacol, in press.

Disclosure: **R. Findling:** Part 1: Abbott, Addrenex, Alexza, AstraZeneca, Biovail, Bristol-Myers Squibb, Forest, GlaxoSmith-Kline, Johnson & Johnson, KemPharm Lilly, Lundbeck, Merck, Neuropharm, Novartis, Noven, Organon, Otsuka, Pfizer, Rhodes Pharmaceuticals, Sanofi-Aventis, Schering-Plough, Seaside Therapeutics, Sepracore, Shire, Solvay, Sunovion, Supernus Pharmaceuticals, Transcept Pharmaceuticals, Validus, Wyeth, Part 2: Shire, Part 4: Abbott, Addrenex, AstraZeneca, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, Lilly, Merck, Neuropharm, Otsuka, Pfizer, Rhodes Pharmaceuticals, Schering-Plough, Shire, Supernus Pharmaceuticals, Wyeth. **E. Youngstrom:** Part 1: Dr. Youngstrom has received travel support from Bristol-Myers Squibb. **N. McNamara:** None. **R. Stansbrey:** None. **C. Demeter:** None. **B. Rowles:** None. **T. Frazier:** Part 1: Dr. Frazier has received federal funding or research support from, acted as a consultant to, or received travel support from Shire Development, Inc., Bristol-Myers Squibb, National Institute of Health, and NARSAD., Part 3: Dr. Frazier has received federal funding or research support from, acted as a consultant to, or received travel support from Shire Development, Inc., Bristol-Myers Squibb, National Institute of Health, and NARSAD., Part 4: Dr. Frazier has received federal funding or research support from, acted as a consultant to, or received travel support from Shire Development, Inc., Bristol-Myers Squibb, National Institute of Health, and NARSAD. **J. Calabrese:** Part 1: Dr. Calabrese has received federal funding or research support from, acted as a consultant to/served on advisory boards for, or provided CME lectures to the Department of Defense, Health Resources Services Administration, National Institute of Mental Health, Abbott, Adamed, AstraZeneca, Bristol-Myers Squibb, Cephalon, Cleveland Foundation, Dainippon Sumitomo, Eli Lilly, EPI-Q, Inc., Eisai, Elan, Forest, France Foundation, Genaissance, GlaxoSmithKline, Janssen, Johnson and Johnson, Jazz Pharmaceuticals, JDS Pharmaceuticals, Lundbeck, Merck, Memory Pharmaceuticals, NARSAD, Neurosearch, Novartis, Organon, OrthoMcNeil, Otsuka, Pfizer, Repligen, Sanofi Aventis, Schering-Plough, Servier, Solvay, Stanley Medical Research Institute, Supernus, Synosia, Takeda, Tikvah, and Wyeth., Part 3: Dr. Calabrese has received federal funding or research support from, acted as a consultant to/served on advisory boards for, or provided CME lectures to the Department of Defense, Health Resources Services Administration, National Institute of Mental Health, Abbott, Adamed, AstraZeneca, Bristol-Myers Squibb, Cephalon, Cleveland Foundation, Dainippon Sumitomo, Eli Lilly, EPI-Q, Inc., Eisai, Elan, Forest, France Foundation, Genaissance, GlaxoSmithKline, Janssen, Johnson and Johnson, Jazz Pharmaceuticals, JDS Pharmaceuticals, Lundbeck, Merck, Memory Pharmaceuticals, NARSAD, Neurosearch, Novartis, Organon, OrthoMcNeil, Otsuka, Pfizer, Repligen, Sanofi Aventis, Schering-Plough, Servier, Solvay, Stanley Medical Research Institute, Supernus, Synosia, Takeda, Tikvah, and Wyeth., Part 4: Dr. Calabrese has received federal funding or research support from, acted as a consultant to/served on advisory boards for, or provided CME lectures to the Department of Defense, Health Resources Services Administration, National Institute of Mental Health, Abbott, Adamed, AstraZeneca, Bristol-Myers Squibb, Cephalon, Cleveland Foundation, Dainippon Sumitomo, Eli Lilly, EPI-Q, Inc., Eisai, Elan, Forest, France Foundation, Genaissance, GlaxoSmithKline, Janssen, Johnson and Johnson, Jazz Pharmaceuticals, JDS Pharmaceuticals, Lundbeck, Merck, Memory Pharmaceuticals, NARSAD, Neurosearch, Novartis, Organon, OrthoMcNeil, Otsuka, Pfizer, Repligen, Sanofi Aventis, Schering-Plough, Servier, Solvay, Stanley Medical Research Institute, Supernus, Synosia, Takeda, Tikvah, and Wyeth.

51. Aripiprazole Augmentation Improves Aspects of Executive Function in Major Depressive Disorder: A Pilot Study

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Background: Cognitive function is a common residual symptom of depression and its effects can be debilitating. One domain of cognitive function, executive functioning, refers to cognitive abilities that involve planning, organization, and manipulation of information and it is among the most consistently noted cognitive deficits associated with depression. Deficient executive functioning can translate into difficulties with everyday tasks that likely contribute to the high degree of psychosocial impairments associated with depression. It is therefore important to identify treatments that specifically target cognitive deficits in depression. This study was conducted to gather preliminary data on the effect of aripiprazole augmentation on cognitive function.

Methods: Seventeen participants with major depressive disorder and residual depressive symptoms following treatment with escitalopram, citalopram, or sertraline, began six weeks of aripiprazole augmentation. Participants had to report difficulties with concentration or cognition and score 2 or greater on the Inventory for Depressive Symptomatology – Clinician-Rated (IDS-C) item measuring this symptom (15: Concentration and Decision Making) to be eligible. Tests from the CAmbridge Neuropsychological Test Automated Battery (CANTAB) were used to assess cognitive function at baseline and post-treatment. Depressive symptoms were measured weekly using the Hamilton Rating Scale for Depression, 17-item version (HRSD) and the 30-item IDS – Self-Report. Psychosocial function was measured at baseline, midpoint, and study end using the Short-Form Health Survey (SF-36), the Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q), and the Work and Social Adjustment Scale (WSAS). Changes in depressive symptom severity and psychosocial function were

assessed via t-tests. Distributions of scores on the cognitive measures were evaluated and due to the non-normal distributions of some measures, a non-parametric Signed Rank test was used to evaluate changes in the cognitive measures. A series of Spearman rank correlations were conducted between the changes in cognitive measures that were significant (pre-post treatment) and changes from baseline to Week 3 and Week 3 to Week 6 on the depressive symptom severity and psychosocial function measures.
Results: Thirteen participants that received 6 weeks of treatment and cognitive testing were included in the analyses.<i > Significant mean reductions in depressive symptom severity were obtained on both clinician-rated (t = 8.79, <i >p<.0001) and self-reported (t = 4.95, <i >p<.0001) measures of depression. Nine (53%) participants responded, defined by a 50% or greater reduction in HRSD score, and seven (41%) remitted, as defined by achieving an HRSD score of 7 or less.<i > Significant improvements in psychosocial function were obtained on the Q-LES-Q (t = 5.84, <i >p = 0.0001) and SF-36 (Mental: t = 3.31, <i >p<.01; Physical = t = 2.40, <i >p<.04) and a trend toward significance was observed on the WSAS (t = 2.21, <i >p<.06). Significant changes on difference scores were found on four cognitive tests, all of which were tests of executive function: the Stockings of Cambridge Mean Initial Thinking Time for 3- (S = −35.5, <i >p<.02) and 5-move (S = −34.5, <i >p<.02) problems, Spatial Working Memory Between Errors for 6-move problems (S = −33.0, <i >p<.01), and Spatial Working Memory Strategy score (S = −23.5, <i >p<.04). Changes in depressive symptom severity occurred primarily early in treatment, with the greatest reductions observed between baseline and Week 3. In contrast, many of the psychosocial function measures showed the greatest improvements in the later weeks of treatment (between Weeks 3 and 6). Interestingly, the majority of higher correlations (.35 or greater) between symptom severity, psychosocial function, and changes in executive function occurred in the last three weeks of treatment, suggesting that cognitive changes are occurring following the greatest reductions in symptom severity and are more strongly associated with the smaller symptom reductions that occur in the last three weeks. In contrast, cognitive changes appear to occur in conjunction with the greatest changes in psychosocial function.
Discussion: This preliminary study indicates that aripiprazole augmentation may improve aspects of cognitive function that are involved in planning and strategic problem-solving. We believe further investigation of the potential benefit of aripiprazole augmentation to improve cognitive function is warranted along with other common residual symptoms that impact quality of life.
Disclosure: T. Greer: None. **P. Sunderajan:** Part 4: Bristol-Myers Squibb; Lilly USA, LLC; Takeda Pharmaceuticals North America, Inc. **B. Grannemann:** None. **M. Trivedi:** Part 1: Abbott Laboratories, Inc.; Alkermes; AstraZeneca; Axon Advisors; Bristol-Myers Squibb Company; Cephalon, Inc.; CME Institute of Physicians; Eli Lilly and Company; Evotek; Forest Pharmaceuticals; GlaxoSmithKline; Johnson and Johnson PRD; Lundbeck; MedAvante; Neuronetics; Otsuka Pharmaceuticals; Pamlab; Pfizer, Inc.; PgxHealth; Rexahn Pharmaceuticals; SHIRE Development; Takeda; Tal Medical/Puretech Venture; Transcept, Part 2: AstraZeneca, Part 4: Naurex; Targacept; Valient.

52. The V1b Receptor Antagonist SSR149415 in the Treatment of Major Depressive and Generalized Anxiety Disorders: Results from Three Double-Blind, Placebo-Controlled Studies

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Background: The vasopressin V1b receptor has been suggested to represent a promising target for the treatment of stress-related disorders. This idea is based on data from studies in animals showing that vasopressin is critical for adaptation of the hypothalamo-pituitary-adrenal (HPA) axis during stress and that the first nonpeptide V1b receptor antagonist SSR149415 exerts anxiolytic- and antidepressant-like effects, findings which have led to this compound being progressed into the clinic.
Methods: Two efficacy and tolerability studies in Major Depressive Disorder (MDD) and one study in Generalized Anxiety Disorder (GAD) were conducted. Studies were randomized 8-week, double-blind, placebo-controlled trials evaluating 100 and 250 mg BID doses of SSR149415, placebo and escitalopram 10 mg or paroxetine 20 mg QD. Patients entered MDD trials with baseline Montgomery-Asberg (MADRS) and Hamilton (HAM-D) Depression Rating Scale total scores $\geqslant$24 and 18, respectively, and the GAD trial with baseline Hamilton Anxiety Rating Scale (HAM-A) total score of $\geqslant$22. Primary efficacy variables included changes from baseline in total score on HAM-D or HAM-A, MADRS, and the secondary variable included changes in severity of illness score on the Clinical Global Impression (CGI). A 4-week, double-blind, placebo-controlled study evaluating the effect of 100 and 250 mg BID doses of SSR149415 on HPA axis in MDD patients was also conducted.
Results: Results showed that in the GAD trial, SSR149415 did not separate from placebo on the primary and secondary outcome measures, while paroxetine demonstrated efficacy with significant separation from placebo. In the first MDD trial, SSR149415-treated patients did not show significant improvement from baseline on any outcome measure compared with placebo-treated patients, unlike paroxetine. However, in a subsequent study in MDD patients, SSR149415 250 mg demonstrated significant improvement compared to placebo on the HAM-D total score at week 8. In all the studies, SSR149415 was safe and well tolerated and had no deleterious effect on the HPA axis at the doses studied.
Discussion: In conclusion, these studies demonstrate that the first non-peptide V1b receptor antagonist, SSR149415, may not be useful for the treatment of GAD and that its antidepressant potential needs to be further evaluated.
Disclosure: G. Griebel: Part 5: Sanofi-Aventis. **S. Stahl:** None. **L. Arvanitis:** Part 5: Sanofi-Aventis.

53. Algorithm-Driven Treatment of Bipolar Disorder in Correctional Setting: Impact on Psychotropic Medication Utilization

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Background: Inmates with bipolar disorder present (BD) a significant challenge for clinicians in the correctional setting due to serious psychiatric/medical comorbidities, substance use and treatment non-compliance. Management of these patients has been associated with inconsistent and disjointed treatment, frequently resulting in polypharmacy and higher costs. Polypharmacy can lead to serious medical consequences (e.g. metabolic syndrome) further increasing the cost burden. Use of evidence-based medication treatment algorithms, as exemplified by the Texas Implementation of Medication Algorithm (TIMA), may facilitate clinical decision-making and optimize pharmacotherapy of BD. We conducted a two-phase study investigating adaptation of the TIMA for BD to the Connecticut correctional setting. The present report delineates impact of TIMA implementation on psychotropic medication utilization and associated costs in this study.
Methods: Study participants included inmates with a diagnosis of BD type I or II without any other predominant psychiatric or medical comorbidities. BD diagnosis was confirmed by means of the Structured Clinical Interview for DSM IV (SCID) in conjunction with a comprehensive interview. TIMA trained clinicians treated enrolled subjects over a 12-week period (bi-weekly visits)

following the TIMA algorithms for BD. In the first, open label phase, a non-randomized sample of 40 inmates (20 males, 20 females) was treated. In the second phase, a total of 61 female inmates were randomized into experimental (TIMA) and treatment as usual (TAU) groups and treated over a 12-week period. Outcome measures ascertained by independent research assistants included bipolar disorder symptoms scale (BDSS), brief psychiatric rating scale, global assessment of functioning, and the short form health survey. Psychotropic medication utilization was assessed at baseline and at the end of 12-week study period. Impact of TIMA driven-treatment on psychotropic medication utilization and associated costs was evaluated in both phases and in patient subpopulations i.e. by gender and by algorithm use. Analysis of the outcome measures was conducted using paired T tests with the last observation carried forward in the intent-to-treat subjects.

Results: In the first phase, a total of 29 subjects completed all the visits and significant improvement was seen with the primary and secondary outcome measures ($p < 0.001$). A sub-analysis showed important differences in BDSS outcomes based on gender (female $p = 0.0002$; male $p = 0.23$) and specific algorithm followed for intervention (mania $p = 0.003$; depression $p = 0.15$). In the second phase, the experimental group ($n = 30$) showed improved outcomes compared to the control group ($n = 31$) but the improvements did not reach statistical significance. At baseline, psychotropic medication utilization was higher in males compared to females including a notable difference in antipsychotic medication use (95% in males vs. 55% in females). In phase I, utilization of antipsychotic and antidepressant medication dropped considerably during the study period, associated with increases in anticonvulsant and anxiolytic medication utilization. In phase II, overall psychotropic medication utilization in the control (TAU) group increased by 19% from baseline to last visit compared to 5% increase in the TIMA group. Notably, the TIMA group showed statistically significant reductions in antipsychotic and antidepressant medication utilization ($p < 0.005$) during the study period compared to the control group. Anticonvulsant medication use increased in the TIMA group compared to the control group.

Discussion: Changes in medication utilization in both phases were consistent with TIMA recommendations and led to considerable reduction in polypharmacy and medication costs as a result of TIMA implementation. Significance of psychotropic medication utilization findings will be discussed in the context of efficacy findings, cost-benefit ratio and medical comorbidities. Benefits and limitations of algorithm-driven treatment of BD in the correctional setting will be discussed in the context of overall study findings and findings in patient subpopulations. The study findings confirmed the feasibility, effectiveness and benefits of TIMA implementation in the correctional setting with critical impact on psychotropic medication utilization. The study findings are being used to optimize TIMA for correctional setting and to inform implementation of evidence-based TIMA-driven treatment of BD throughout the Connecticut correctional facilities.

Disclosure: J. Kamath: None. **S. Wakai:** None. **W. Zhang:** None. **R. Trestman:** None.

54. Early Improvement as a Predictor of Later Treatment Response in Acute Manic or Mixed Episodes using CGI Assessments: A Pooled, Post-Hoc Analysis

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Background: Early symptomatic improvement, measured as changes of the Young Mania Rating Scale (YMRS) total score, has been reported to be a clinically useful indicator of later treatment outcome in manic or mixed episodes in bipolar disorder. In clinical practice, routine ratings using the YMRS scale can be time and resource intensive. We sought to investigate whether the brief overall assessment of the severity of illness on the Clinical Global Impression scale either related to severity of bipolar mania [CGI-BP mania], or to overall severity of illness [CGI- BP overall illness]) and its changes during treatment could have similar predictive value.

Methods: Data were pooled from the intent-to-treat populations of two 3-week randomized, double-blind trials [NCT00159744 and NCT00159796]. Patients were administered flexible-dose sublingual asenapine (5 or 10 mg twice daily; $n = 372$), oral olanzapine (5–20 mg once daily; $n = 391$), or placebo ($n = 197$). Analysis was done based on CGI-BP severity of mania or CGI-BP severity of overall illness scores. Early improvement, defined as reduction from baseline CGI (baseline $\geqslant 4$) by at least 1 point, was assessed in each patient at days 2, 4 and 7. Treatment response was assessed at Week 3 and defined as a score of "minimally ill" or "not at all ill". Remission was defined as "not at all ill". Associations between early improvement and later treatment outcome were calculated using Fisher exact tests; odds ratios classified their relative strength. Sensitivity (SN), specificity (SP), and positive (PPV) and negative (NPV) predictive values were also calculated. In the data presentation, missing treatment outcomes for individual patients were treated as treatment failures.

Results: Early improvement was positively associated with treatment outcome. For CGI-BP severity of mania, the earliest positive associations were observed with asenapine at day 2 for both CGI-BP mania response ($P < 0.03$) and CGI-BP mania remission ($P < 0.01$), olanzapine at day 4 for response ($P < 0.02$) and remission ($P < 0.01$), and placebo on day 7 for response ($P < 0.001$) and remission ($P < 0.02$). For CGI-BP severity of overall illness, the earliest positive associations were observed with asenapine at day 4 for both CGI-BP overall illness response ($P < 0.0001$) and CGI-BP overall illness remission ($P < 0.0001$), with olanzapine at day 4 for overall illness response ($P < 0.02$) and overall illness remission ($P < 0.04$), and placebo on day 7 for response ($P < 0.001$) and remission ($P < 0.01$). Odds ratios for early improvement at day 2, 4 and 7 leading to later positive mania response were higher for asenapine (2.0, 5.1, 7.2) than for olanzapine (1.4, 1.8, 3.8) and placebo (1.2, 1.2, 5.8). Respective mania response values for SN, SP, PPV, and NPV at day 4 were 71%, 68%, 42%, and 88% for asenapine; 58%, 56%, 35%, and 77% for olanzapine; and 29%, 75%, 20%, and 83% for placebo. Odds ratios for early improvement at day 2, 4, and 7 leading to later positive mania remission were higher for asenapine (3.0, 11.8, infinite) than for olanzapine (1.8, 2.9, 4.5) and placebo (0.9, 1.3, 6.0); Respective remission values for SN, SP, PPV, and NPV at day 4 were 88%, 63%, 19%, and 98% for asenapine; 71%, 54%, 13%, and 95% for olanzapine; and 30%, 75%, 7%, and 95% for placebo. Odds ratios for early improvement leading to positive outcome on overall illness yielded similar results to those seen in mania. These figures closely resemble the respective figures previously calculated for the YMRS scale (Zhao *et al*., 2010).

Discussion: In acute manic or mixed episodes, early improvement during monotherapy with asenapine, olanzapine, and placebo was strongly associated with CGI-BP response and remission at week 3. Presence of early improvement on the CGI scale indicated a significantly increased chance of response or remission at the end of treatment. Conversely, if early improvement was absent, the chances of later response or remission were low, as indicated by the high NPV. The predictive value of the simple CGI assessment appeared to resemble the predictive value for the more detailed and time consuming YMRS assessments. These data indicate that a simple CGI assessment can be used by clinicians to tailor individual treatment as early as day 2-4 with asenapine or olanzapine.

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Disclosure: A. Szegedi: Part 1: Full time employee at Merck, Part 2: Full time employee at Merck, Part 3: Full time employee at Merck, Part 4: Full time employee at Merck, Part 5: Merck. **C. Karsson:** Part 1: Full time employee at Merck, Part 2: Full time employee at Merck, Part 3: Full time employee at Merck, Part 4: Full time employee at Merck, Part 5: Merck. **J. Zhao:** Part 1: Full time employee at Merck, Part 2: Full time employee at Merck, Part 3: Full time employee at Merck, Part 4: Full time employee at Merck, Part 5: Merck.

55. Efficacy and Safety of Lisdexamfetamine Dimesylate in Adults with Executive Dysfunction and Partial or Full Remission of Major Depressive Disorder

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Background: Executive dysfunction in major depressive disorder (MDD) has been reported, even during remission. Dopaminergic modulation may have therapeutic efficacy for executive dysfunction in MDD. We evaluated adjunctive lisdexamfetamine dimesylate (LDX) to antidepressant monotherapy for executive dysfunction in MDD.

Methods: Participants (18–55 y) in this randomized, placebo-controlled, multicenter study were on stable antidepressant monotherapy, had *DSM-IV-TR*–diagnosed MDD of mild severity (Montgomery-Asberg Depression Rating Scale [MADRS] total score $\leqslant$18 at screening and baseline), and executive dysfunction as indicated by a global composite T-score of $\geqslant$60 on the Behavioral Rating Inventory of Executive Function–Adult Version (BRIEF-A). After a 2-week screening period, participants were randomized to double-blind LDX treatment (week 1: 20 mg/d; weeks 2–6: maintain or increase LDX in 10-mg increments weekly to 70 mg/d; weeks 7–9: maintain optimized LDX dose) or placebo. The 9-week double-blind treatment was followed by a 2-week single-blind phase. The primary efficacy endpoint was mean change (baseline to week 9) on the BRIEF-A Global Executive Composite T-score. Secondary assessments included MADRS score changes. Statistical analyses of efficacy used analysis of covariance with last observation carried forward. Safety assessments included treatment-emergent adverse events (TEAEs) and vital sign assessments.

Results: Of 143 randomized participants (LDX, n = 71; placebo, n = 72), 119 completed double-blind treatment (LDX, n = 60 [84.5%]; placebo, n = 59 [81.9%]). Mean ± SD baseline BRIEF-A Global Executive Composite was 76.8 ± 9.66 for LDX and 74.2 ± 8.88 for placebo; baseline MADRS total scores were 12.7 ± 3.23 and 11.8 ± 3.77 for LDX and placebo, respectively. At week 9, mean reductions from baseline were significantly greater for LDX versus placebo for BRIEF-A Global Executive Composite score (least squares mean [95% CI] change: −21.2 [−24.5, −17.9] vs −13.2 [−16.5,−9.9]; $P = 0.0009$). Significantly greater improvements for LDX versus placebo were also observed for MADRS total score (least squares mean [95% CI] change: −5.0 [−6.3, −3.6] vs −3.1 [−4.4, −1.8], $P = 0.0465$) at week 9. TEAEs led to discontinuation in 5 participants (LDX, 4; placebo, 1). The most frequent TEAEs (incidence 10%) were decreased appetite (22.5%), headache (22.5%), dry mouth (15.5%), insomnia (14.1%), and irritability (12.7%) with LDX and headache (15.3%) with placebo. Increases in blood pressure and heart rate with LDX were relatively modest.

Discussion: Adjunctive LDX treatment significantly improved executive dysfunction (assessed by BRIEF-A) and depressive symptoms in MDD participants with an incomplete response to antidepressant monotherapy. The safety profile of LDX was generally consistent with prior studies. (Supported by Shire Development, Inc.)

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56. Baseline Interleukin-6, Cortisol, and Insulin in Major Depressive Disorder and Response to Pioglitazone: Preliminary Support for Insulin Sensitizers as Modulators of Mood

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Background: Insulin resistance occurs 2- to 3-fold more commonly among individuals with depression than in the general population. Cross-sectional and prospective studies suggest that insulin resistance is significantly associated with depression severity and may be causally related to the development of depression. Supportive of an overlapping pathophysiology, modulation of insulin sensitivity affects several pathways that regulate mood and behavior, including inflammatory networks, neuroendocrine stress markers, adipose-derived hormones, and monoamine transporters (Kemp *et al.*, 2011). Pre-clinical evidence suggests the insulin-sensitizer, pioglitazone, has antidepressant-like effects and can attenuate neurotoxicity within the central nervous system. The objective of this study was to test the feasibility of pioglitazone as an antidepressant treatment in humans with Major Depressive Disorder (MDD) and co-occurring metabolic syndrome or abdominal obesity and to identify potential biomarkers associated with greater antidepressant response. Eventually, if proven safe and effective, pioglitazone may replace antidepressant treatments that worsen insulin resistance or dysplipidemia and generate insight into non-conventional antidepressant mechanisms of

action that act by reducing inflammation or altering insulin signaling.

Methods: Forty patients with a DSM-IV diagnosis of MDD as ascertained by the Mini International Neuropsychiatric Interview and currently experiencing an acute major depressive episode received 12 weeks of open-label pioglitazone initiated at 15mg daily for 4 weeks followed by titration to a maximum of 45 mg daily. Pioglitazone was administered as monotherapy ($n = 9$) or in augmentation to conventional antidepressants ($n = 31$). No change in concurrent antidepressant therapy was permitted for up to 4 weeks prior to enrollment or during the trial. Severity of mood symptoms was rated using the clinician-administered Inventory of Depressive Symptoms (IDS) and patient-administered Quick Inventory of Depressive Symptoms (QIDS).

Results: Participants were moderately to severely depressed at study entry (baseline mean IDS total score $= 38.7 \pm 6.4$) and 60% ($n = 24$) had failed 2 or more antidepressants during the current episode. Pioglitazone was associated with a significant reduction in depression severity according to both the IDS (-19.9 ± 1.9; $p < .001$) and QIDS (-7.3 ± 1.0; $p < .001$). Significant improvement also occurred in several inflammatory and cardiometabolic parameters, including highly-sensitive C-reactive protein (-3.3 mg/L $\pm$ 1.0; $p = 0.002$), fasting triglycerides (-33.9 mg/dL $\pm$ 10.9; $p = 0.004$), fasting glucose (-10.1 mg/dL $\pm$ 2.7; $p = 0.008$), and insulin resistance as measured by homeostasis model assessment (HOMA-IR) (-2.7 ± 0.7; $p < .001$). Inferential analysis of hypothesized outcome predictors suggested a greater therapeutic effect size (Cohen's d) on IDS change scores among patients in the upper tertile of IL-6 (-26.2 vs. -17.7; $d = 0.78$) and cortisol (-24.6 vs. -17.7; $d = 0.64$) at baseline. The effect size for total change in QIDS scores was greater among patients with fasting insulin levels below the median of 15 uIU/ml (-9.9 vs. -6.7; $d = 0.62$). Linear regression identified potential mediators of mood improvement, such that change in IL-6 at week 4 was associated with baseline to endpoint change in IDS total score ($p = 0.055$). Improvement in depressive symptoms was positively correlated with a reduction in IL-6 ($r = 0.37$; $p = 0.059$). Pioglitazone was well tolerated, and no serious adverse events occurred during the trial.

Discussion: These preliminary efficacy and safety data demonstrate evidence of feasibility for pioglitazone to reduce depression severity and suggest that patients with metabolic syndrome or abdominal obesity may experience benefits in both mood and cardiometabolic health with pioglitazone treatment. Prior studies have found the efficacy of conventional antidepressant treatments to be reduced in the presence of co-occurring cardiometabolic disorders. Consistent with this observation, the treatment effect size was smaller among patients with higher baseline insulin levels. In contrast, elevated IL-6 and cortisol may represent candidate peripheral biomarkers for good outcome after treatment with pioglitazone for acute depression. A significant correlation between change in IL-6 and change in IDS total score lends support to mitigation of inflammation as a mediator of improved mood. The results of this study need to be confirmed over a longer duration and in larger placebo-controlled clinical trials.

Disclosure: D. Kemp: Part 1: AstraZeneca Bristol-Myers Squibb Pfizer, Part 2: AstraZeneca Pfiaer, Part 3: AstraZeneca Pfzier. **S. Ganocy:** Part 4: AstraZeneca Eli Lilly. **K. Gao:** Part 1: AstraZeneca, Pfizer, Part 2: N/A, Part 3: N/A, Part 4: AstraZeneca, Part 5: N/A. **F. Ismail-Beigi:** Part 1: Eli Lilly. **C. Conroy:** None. **S. Obral:** None. **J. Calabrese:** Part 1: Abbott AstraZeneca Bristol Myers Squibb Cephalon Dainippon Sumitoma Forest Glaxo-SmithKline Jannsen Johnson & Johnson Lundbeck Merck Otsuka Pfizer Schering-Plough Servier Supernus Takeda Wyeth, Part 2: 2009 - AstraZeneca, GlaxoSmithKline, Wyeth 2010 - AstraZeneca, Merck 2011n - AstraZeneca, Part 3: AstraZeneca, Part 4: AstraZeneca Cephalon Dainippon Sumitomo.

57. Bipolar Clinical and Health Outcomes Initiative in Comparative Effectiveness (Bipolar CHOICE): Rationale and Design

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Background: Pharmacotherapy of bipolar disorder (BD) has changed considerably over the last decade, withthe prototypal classic mood stabilizer lithium(Li) being largely replaced with alternate, commercially promoted anticonvulsants and second-generation antipsychotics (SGAs) that may or may not yield improved effectiveness. The SGA mood stabilizer quetiapine (QTP) is the only one of these newer agents to receive United States Food and Drug Administration approval for all phases of BD (acute mania, acute depression and maintenance treatment) and is anticipated to be available in a generic formulation by approximately March 2012. No study has compared the effectiveness of Li-based treatment with QTP-based treatment.

Methods: Bipolar CHOICEwill randomize 480 adults with BD (Type I or II) across 10 study sites to Li plus adjunctive personalized treatment (APT) to manage specific mood states and comorbid conditionsversus QTP + APTfor six months. Those in the Li + APT group may not be treated with any antipsychotic drug and those in the QTP + APT group may not be treated with Li. APT can include any other medications needed to manage specific mood states and comorbid conditions. The co-primary outcomes are overall bipolar illness severity in relationship to side effects using the single-blind rated clinical global impression efficacy index (CGI-EI) and a novel measure, necessary clinical adjustments (NCAs). The CGI-EI integrates benefits and harms and yields a score that can be compared across interventions. NCAs represent a count of the clinician's recommended medication adjustments to reduce symptoms, optimize response and functioning, or to address intolerable side effects. Secondary outcomes include the future risk of cardiovascular diseaseusing the Framingham General Cardiovascular Risk Score, functional status as measured by the Longitudinal Interval Follow up Evaluation Range of Impaired Functioning Tool (LIFE-RIFT), and potential baseline moderators of response with regard to demographic variables, symptomatic course (i.e. younger age, ethnic minority, comorbid psychiatric disorders, especially anxiety, and comorbid medical disorders).

Results: As of August 18th, 2011, 228 patients have been consented and 160 randomized.

Discussion: The strengths of the study include a generalizable group of participants with a full range of comorbid conditions, 6-month outcomes, and a direct comparison of benefit and harm. Moderator analyses could help identify optimal patient profiles for treatment with Li or QTP. The potential limitations of Bipolar CHOICEinclude lack of a placebo-control group, open randomized treatment, and use of a novel co-primary outcome measure (i.e. NCAs). We expect this study will inform our understanding of a novel complex comparative effectiveness design that compares Li versus QTP as foundational mood stabilizers for the treatment of BD.

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Astra Zeneca, Eli Lilly, and Bristol-Myers Squibb. No speaker bureaus or boards since 2003. Dr. Nierenberg owns stock options in Appliance Computing, Inc. and Brain Cells, Inc. Additional income is possible from Infomedic.com depending on overall revenues of the company but no revenue has been received to date. Through MGH, Dr. Nierenberg is named for copyrights to: the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery Asberg Depression Scale exclusively licensed to the MGH Clinical Trials Network and Institute (CTNI)., Part 2: None., Part 3: None., Part 4: Dr. Nierenberg has received grant/research support through MGH from AHRQ, Cephalon, NIMH, PamLabs, Pfizer Pharmaceuticals, and Shire. In the next 2 years, it is possible that he will receive grants from Dey Pharmaceuticals, Sunovion, and Targacept., Part 5: Not Applicable.

58. Efficacy of Adjunctive Aripiprazole in Patients with Major Depressive Disorder whose Symptoms Worsen with Antidepressant Monotherapy: A Pooled Analysis of Three Trials

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Background: More than half of patients with major depressive disorder (MDD) initially treated with antidepressant therapy (ADT) do not achieve an adequate response or remission.[1] For these patients, current American Psychiatric Association guidelines[2] recommend considering augmentation with another agent or switching to a second ADT. A previous meta-analysis found that augmentation of ADT with atypical antipsychotics was effective in treatment-resistant depressed patients.[3] Clinicians, however, have favored switching ADT over augmentation for patients who do not achieve at least a partial response.[4] Patients who show no response or worsen during initial ADT are especially likely to be switched. In this *post-hoc* analysis, we evaluated pooled data from three similarly designed, randomized, double-blind, placebo-controlled trials of adjunctive aripiprazole in patients with MDD, who had undergone a prospective period of ADT monotherapy and whose symptoms increased. To our knowledge, this is the first attempt to assess the efficacy of an adjunctive intervention for this difficult-to-treat MDD population.
Methods: This *post-hoc* analysis pooled data from three nearly identical studies of adjunctive aripiprazole of ADT. Eligible patients were aged 18–65 years and diagnosed with a major depressive episode (Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision) lasting $\geqslant$8 weeks and 17-item Hamilton Rating Scale for Depression (HAM-D17) total score $\geqslant$18 at baseline. Patients had an inadequate response to one to three adequate prior ADT trials during the current episode. Patients were enrolled in an 8-week, prospective ADT monotherapy phase. The patients with an inadequate response to the prospective ADT monotherapy then entered a 6-week, randomized, double-blind, placebo-controlled phase. Aripiprazole dosing was started at 5 mg/day and was increased to 10 mg/day at the end of Week 1 if tolerated, with a maximum dose of 15–20 mg/day. The current analysis examined the subset of patients who showed an increase in MADRS during the ADT monotherapy phase. Change on the Montgomery–Åsberg Depression Rating Scale (MADRS), response rates ($\geqslant$50% reduction in MADRS), and remission rates ($\geqslant$50% reduction in MADRS total score and MADRS total score $\leqslant$10) were evaluated in this subpopulation.
Results: The subpopulation comprised 160 patients: 71 randomized to aripiprazole and 89 to placebo. Patients entered ADT monotherapy with a baseline MADRS score of 28. The mean increase in MADRS score during ADT monotherapy was 13.3% (range 3.0–52.6%). During adjunctive treatment, MADRS scores improved significantly more from adjunctive baseline to endpoint with adjunctive aripiprazole (–12.0) compared with placebo (–8.7; $p = 0.03$). Significant differences between the two groups were observed from Week 3 onward. At endpoint, 36.6% of the aripiprazole group and 22.5% of the placebo group responded ($p = 0.06$). Significantly more patients remitted with adjunctive aripiprazole vs. placebo in the final 2 weeks (21.1% vs. 7.9%, respectively, $p = 0.02$ at Week 5; and 25.4% vs. 12.4% respectively, $p = 0.04$ at Week 6 endpoint). Adverse event data are also reported.
Discussion: These results indicate that adjunctive aripiprazole may be an effective intervention even in patients whose symptoms worsen during ADT monotherapy, and suggest an alternative strategy for clinicians. The results also have interesting neuropharmacologic implications. Because 8 weeks of ADT had no clinical effect, the initial treatment must have either "primed the pump", resulting in a synergistic effect when aripiprazole was added, or alternatively, aripiprazole has independent antidepressant effects. This early onset of adjunctive aripiprazole efficacy (beginning at Week 3) is clinically valuable to both clinicians and patients in a difficult-to-treat population and allows treatment adjustments (e.g. dose increases) early in the course of therapy.
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59. A Prospective Study of the Role of Life Events in Precipitating Suicidal Behavior

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Background: Suicidal behavior is often conceptualized as a response to overwhelming stress. Our stress-diathesis model posits that suicide attempts occur among individuals with a propensity for acting on suicidal urges (the diathesis) precipitated by stressors such as life events or a major depressive episode (MDE). We hypothesized that recent life events (RLEs) as measured by the

Recent Life Changes Questionnaire (RLCQ), and MDEs would precipitate suicidal behavior in mood disordered individuals, independently of the presence of a diathesis.
Methods: Depressed patients (n = 430) were followed for two years post-discharge while receiving naturalistic treatment in the community. Participants were assessed prospectively for mood symptomatology, occurrence of life events and recurrence of MDE. Using the Andersen-Gill extension to a Cox proportional hazards regression model with MDE status and life event score as time-varying covariates, we controlled for baseline characteristics linked to presence of a diathesis for suicidal behavior. Suicidal behavior was the outcome variable.
Results: Epochs during the follow-up period were defined as having constant MDE and RLE status, uninterrupted by suicide attempt. A longitudinal Cox regression model predicted future suicide attempt during the follow-up period ($p \leq 0.0005$). While MDE recurrence increased the risk nearly five-fold [HR = 4.90 (95% C.I.: 2.52, 9.52); $p < 0.001$], life events did not appear to precipitate suicidal behavior [HR = 1.02 (95% C.I.: 0.94, 1.14); $p < 0.6503$]. Even in the context of an MDE, life events had no effect. Among baseline characteristics, sex [HR = 0.36 (95% C.I.: 0.14, 0.90); $p < 0.0294$] and one of two baseline pessimism factors [HR = 1.54 (95% C.I.: 1.05, 2.26); $p < 0.0283$] predicted suicide attempts.
Discussion: Although the role of stressful life events in precipitating suicidal acts requires further study, these data undermine clinical lore positing a relationship between life events and suicidal acts. These findings parallel recent reports for mood disorders suggesting that "reasons" for depression, which make the occurrence of symptoms "understandable" may not in fact be the <i>causes of depression. Of note, recurrent MDE is a strong predictor of suicide attempts. That a treatable risk factor such as depression so solidly predicts suicide attempts is cause for hope. Interventions to minimize MDE recurrence should be a priority in suicide prevention efforts.
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60. Efficacy of Vilazodone in Patients with Moderate, Moderately Severe and Severe Depression - Pooled Analyses from 2 Randomized Phase III Trials

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Background: Major depressive disorder (MDD) is a common and debilitating illness. Increased severity of depression is accompanied by greater social and functional impairment, higher levels of morbidity and mortality, longer duration of illness with less likelihood of spontaneous remission, and greater risk of relapse. Vilazodone, a serotonin reuptake inhibitor and 5-HT_{1A} receptor partial agonist, is approved by the US Food and Drug Administration for the treatment of MDD. Post hoc analyses were performed evaluating the efficacy of vilazodone in depression severity subgroups using data from placebo-controlled phase III trials, both of which were positive.
Methods: Data from 2 phase III 8-week, double-blind, randomized, placebo-controlled trials (NCT00285376, NCT00683592) were pooled to analyze the effects of vilazodone. The trials comprised patients age 18-70 years with DSM-IV TR-defined MDD and a minimum score ≥ 22 on the 17-item Hamilton Depression Scale. Study designs were similar in both trials with a 1-week screening period followed by 8-weeks double-blind treatment. Patients randomized to vilazodone were titrated to a target dose of 40 mg once daily over a 2-week period according to a fixed-titration schedule (10 mg once daily (qd) for 7 days, 20 mg qd for the next 7 days, and 40 mg qd thereafter). Efficacy outcome (mean change from baseline in Montgomery-Asberg Depression Rating Scale [MADRS] at end of treatment [EOT], the primary efficacy variable in the trials) was assessed using an Analysis of Covariance (ANCOVA) model based on the intent-to-treat population (ITT, patients receiving study medication with post-baseline efficacy evaluation) with missing values imputed by the last observation carried forward (LOCF) method. Subgroup analyses involved patients categorized by baseline depression severity according to MADRS threshold scores: moderate depression ($MADRS < 30$), moderately severe depression ($30 \leq MADRS < 35$), and severe depression ($MADRS \geq 35$).
Results: Of 863 patients (ITT population), baseline MADRS score classified 31% with moderate (placebo, n = 143; vilazodone, n = 128), 49% with moderately severe (placebo, n = 204; vilazodone, n = 217) and 20% with severe depression (placebo, n = 85; vilazodone, n = 86). In the 863 ITT population (placebo, n = 432; vilazodone, n = 431), least-square mean difference (LSMD) for change from baseline in MADRS total score at EOT was significantly better for vilazodone versus placebo (LSMD = −2.8 [95% CI = −4.1, −1.4]; $P < .0001$). A similar magnitude of improvement was observed for vilazodone relative to placebo in each of the three subgroups, with no obvious trend with increasing severity: $MADRS < 30$ (LSMD = −2.9 [95% CI = −5.0, -0.9]; $P = 0.0056$), $30 \leq MADRS < 35$ (LSMD = −2.3 [95% CI = −4.4, -0.2]; $P = 0.0314$), and $MADRS \geq 35$ (LSMD = −4.1 [95% CI = −7.4, -0.7]; $P = 0.017$).
Discussion: In these post hoc pooled analyses of 2 pivotal phase III studies, vilazodone showed significantly greater improvement in MADRS scores compared with placebo treatment overall, as well as in patient subgroups with moderate, moderately severe, and severe depression. The mean differences in MADRS change from baseline versus placebo were >2.0 in all three subgroups and the ITT population, a treatment effect considered clinically significant.
Disclosure: D. Robinson: Part 1: Consultant, Dey Pharma, Forest Research Institute. **C. Reed:** Part 5: Forest Research Institute. **W. Song:** Part 5: Forest Research Institute. **J. Edwards:** Part 5: Forest Research Institute. **P. Ross:** Part 5: Forest Research Institute.

61. Comparison of Computer vs Site-based Rater Administration of the MADRS in Three Placebo Controlled Trials

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Background: Determining the reliability of clinical rating scales across the range of circumstances of its proposed use is key to establishing the acceptability of any measure proposed as a drug development tool. Since most reports establishing scale and rater reliability derive their data from an exercise conducted apart from an actual clinical trial, there is a need to establish the performance characteristics of scales within the context of their intended use. Well established scales such as the Montgomery Asberg Depression Rating Scale (MADRS) may provide a useful benchmark against which to judge the merit of other proposed measures.
Methods: Three multicenter placebo controlled double blind studies were identified in which the MADRS was administered independently by site based raters ($MADRS_{SBR}$) and by a computer ($MADRS_{COMP}$) as part of a quality management program. Internal scale consistency was assessed using Cronbach's alpha calculated for the $MADRS_{SBR}$ and $MADRS_{COMP}$ at baseline, the first post randomization visit, study endpoint, other visits, and all visits.

Table 1 Computer and Site-Based Rater Scale Performance across study visits

	Baseline Cronbach's Alpha N Mean (S.D)	First post randomization visit Cronbach's Alpha N Mean (S.D)	Study end point (last visit) Cronbach's Alpha N Mean (S.D)	Other Visits Cronbach's Alpha N Mean (S.D)	All Visits Cronbach's Alpha N Mean (S.D)
Overall $MADRS_{SBR}$	0.674	.809	.891	.879	.889
	1020	936	686	4732	7544
	30.3(5.8)	24.4 (8.2)	14.3(10.4)	17.8 (10.2)	20.1(10.7)
Overall $MADRS_{COMP}$	.688	.776	.854	.841	.847
	1020	936	686	4732	7544
	30.4(8.6)	23.4 (10.2)	15.0 (11.6)	18.1(11.5)	20.3(11.9)

All ratings were made in the subject's native language. Comparisons of the $MADRS_{SBR}$ and $MADRS_{COMP}$ were made at overall and at four study time points Baseline, First Post-randomization visit, Study Endpoint and all other visits. Correlations between the pairs were calculated to examine measurement reliability overall and individually for each MADRS item.
The variance observed by site based rater as compared to that observed by the computer at each time point.
Results: The sample included 7544 pairs of $MADRS_{SBR}$ and $MADRS_{COMP}$ ratings. The datasets had no subject identifiers other than the subject's study identification number.
Discussion: Guidance on the development of drug development tools makes clear the need for scale performance data. This analysis shows good reliability for the MADRS at all study visits after baseline. Close agreement between $MADRS_{COMP}$ and $MADRS_{SBR}$ across study visits and therapeutic indications during actual clinical trials supports the validity and reliability of computer-administered MADRS in studies of Unipolar and Bipolar Depression. The $MADRS_{SBR}$ and $MADRS_{COMP}$ may be a useful benchmarks against which to judge the performance of other scales proposed for use in global multisite studies. The similar drop in performance observed for both the $MADRS_{SBR}$ and $MADRS_{COMP}$ at study baseline visits suggests that factors other than rater behavior may play an important role undercutting the reliability of baseline scores. Strengths and weaknesses of $MADRS_{SBR}$ and $MADRS_{COMP}$ will be considered.
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62. Suicide, Depression, and Complicated Grief

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Background: Complicated grief(CG) is a debilitating syndrome being considered for inclusion in DSM5 and ICD11. People with CG feel stuck in their grief, experiencing prolonged intense sadness, yearning and longing for the deceased, preoccupation with thoughts of the person who died, avoidance of reminders of the loss and difficulty finding meaning or purpose in life without the deceased. CG occurs in about 6% of bereaved people overall, with higher rates (e.g. 20%) reported after suicide. It is not known if there are clinically significant differences in CG following suicide. Analyses reported here compare suicide to non-suicide bereaved participants in an ongoing NIMH and AFSP-funded multisite clinical trial.
Methods: 23 suicide bereaved and 139 non-suicide bereaved participants with CG, randomly assigned to treatment with citalopram alone, placebo alone, citalopram + complicated grief therapy (CGT) or placebo + CGT, are compared using two-sample t-test for continuous outcomes or Fisher's exact test for categorical outcomes. We describe baseline characteristics of the sample to examine whether CG following suicide bereavement differs from CG following non-suicide bereavement and to determine rates of suicidal ideation and behavior in each group.
Results: Suicide bereaved participants are younger (48 years ± 16 v 54 years ± 14; t = 2.11, p = 0.04), less likely to have CG due to loss of a partner (19% v 38%) or a parent (19% v 33%), and more likely due to loss of a child (26% v 13%) or other relative or friend (26% v 13%) (Fischers exact test of relationship to deceased, p = 0.009.) There are no differences in education level, employment rate, mean time since the death (5.2 ± 2.2 v 4.5 ± 2.8 years) or level of perceived social support. CG symptoms: Suicide bereaved participants report more severe impairment from grief (mean work and social adjustment scale 27.0 ± 9.4 v 22.0 ± 10.6; t = -2.11, p = 0.04), experience pain in the same area of their body more often (p = .006, Fisher's exact test) and believe more strongly that their loved one did not have to die this way (p = 0.008, Fisher's exact test.) However, there are no group differences on CG symptom severity as measured by the Inventory of Complicated Grief or the clinical global impression severity scale, and little differences in severity or pattern of grief-related avoidance or grief-related beliefs. Comorbidity: Suicide bereaved participants are more likely to meet criteria for lifetime (68% v 38%; p = 0.008; Fisher's exact test) and current (56% v 31%; p = 0.02; Fisher's exact test) major depression, with no differences in rates of panic disorder, GAD or PTSD. Suicidality: 44% of suicide bereaved v 30% of non-suicide bereaved reported having suicidal ideation before the CG-inciting death; 9% of suicide bereaved and 11% of non-suicide bereaved reported suicide intent, with or without a plan and 9% of suicide and 7% of non-suicide bereaved made a suicide attempt before the death. 61% of suicide bereaved and 57% of non-suicide bereaved had suicidal thoughts after the death; 13% v 8% reported intent respectively(p = 0.11, Fisher's exact test) and 4% of suicide bereaved v 2% of non-suicide bereaved made an attempt after the CG-inciting death. Before the CG-related death, 60% of all participants with suicidal ideation wanted to die to end the pain they were in. Only one (non-suicide bereaved) wanted to die because of loss of a loved one. After the CG death 40% of non-suicide bereaved wanted to find or join the deceased or felt guilty living when this person died, compared to only one suicide bereaved (p = 0.009, Fisher's exact test.)

Discussion: Suicide bereaved participants in a complicated grief treatment study are younger and more likely to have CG after losing someone other than a parent or life partner. The severity and pattern of CG symptoms is remarkably similar in both groups, as is the frequency of anxiety disorder comorbidity, including PTSD. However, suicide bereaved participants have almost twice the rate of lifetime and current major depression. In spite of this, there are no significant differences in rate of suicidal thinking or behavior before or after the death. Notably, suicidal thoughts are common in both CG groups after the CG-related death, and should be taken seriously. Clinicians treating CG following suicide bereavement should be alert to high rates of major depression and suicide risk. CG in non-suicide bereaved is also associated with marked increase in frequency of suicidal thinking, only slightly less pronounced than in suicide bereaved.
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63. Oxcarbazepine for Acute Affective Episodes of Bipolar Disorder: A Cochrane Review and Meta-analysis

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Background: Oxcarbazepine, a keto derivative of the 'mood stabiliser' carbamazepine, may have efficacy in the treatment of acute episodes of bipolar disorder. Potentially, it may offer pharmacokinetic advantages over carbamazepine.
Methods: To review the efficacy and acceptability of oxcarbazepine compared to placebo and other agents in the treatment of acute bipolar episodes including mania, mixed episodes and depression. *Search Methods:* Electronic database of the Cochrane Central Registration of Controlled Trials (CENTRAL) were searched till December 2010. Specialist journals and conference proceedings were hand-searched. Authors, experts in the field and pharmaceutical companies were contacted requesting information on published and unpublished trials. *Selection Criteria:* Randomised controlled trials (RCTs) which compared oxcarbazepine with placebo or alternative agents, where the stated intent of intervention was the acute treatment of bipolar affective disorder were sought. Bipolar patients of either sex and of all ages were included. *Data Collection and Analysis:* Data were extracted from the original reports individually by two reviewers. For dichotomous data, odds ratios (ORs) were calculated with 95% confidence intervals (CI). Continuous data were analysed using standardised mean differences (with 95% CI).
Results: Six studies were included in the analysis. All were on mania, hypomania, mixed episodes or rapid-cycling disorder. Overall, their methodological quality was relatively poor.
There was no difference in the primary outcome analysis –'a fall of 50% or more on the Young Mania Rating Scale (YMRS)' -between oxcarbazepine and placebo (N = 1, n = 110, OR = 2.10, 95% CI 0.94 to 4.73) in one study conducted in children. In comparison with other mood stabilisers, there was no difference between oxcarbazepine and valproate as an antimanic agent using the primary outcome (50% or more fall in YMRS, OR = 0.44, 95% CI 0.10 to 1.97, 1 study, n = 60, p = 0.273) or the secondary outcome measure (differences in YMRS in the two groups, SMD = 0.18, 95% CI -0.24 to 0.59, 2 studies, n = 90, p = 0.40). No primary or secondary efficacy outcome measures were found comparing oxcarbazepine with lithium monotherapy. As an adjunctive treatment to lithium, oxcarbazepine reduced depression rating scale scores more than carbamazepine in a group of manic patients on MADRS (SMD = − 1.12, 95% CI -1.71 to -0.53, 1 study, n = 52, p = 0.0002) and on the HDRS (SMD = − 0.77, 95% CI -1.35 to -0.20, 1 study, n = 52, p = 0.008)
There was a higher incidence of adverse effects, particularly neuropsychiatric, in patients randomised to oxcarbazepine compared to those on placebo (1 study, n = 115, 17% to 39% of participants on oxcarbazepine had at least one such event compared to 7% to 10% on placebo).There was no differerence in adverse events rates between oxcarbazepine and other mood stabilisers or haloperidol.
Discussion: Currently, there are insufficient trials of adequate methodological quality on oxcarbazepine in the acute treatment of bipolar disorder to inform us on its efficacy and acceptability in this context. Studies predominantly examine the treatment of mania: there are data from sub-group analyses on mixed affective episodes, hypomania and rapid-cycling disorder. From the few studies included in this review, oxcarbazepine did not differ in efficacy compared to placebo in children and adolescents. It did not differ from other active agents in adults. It may have a poorer tolerability profile compared to placebo. No data were found on outcomes relevant to patients and clinicians, such as length of hospital admission.
There is a need for adequately powered randomised controlled trials of good methodological quality to inform us of the therapeutic potential of oxcarbazepine across the spectrum of acute episodes in bipolar disorder.
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64. Do Current Internet-Based Patient Recruitment Methods Impact Patient Retention and Completion Rates in Depression Studies and Are These Metrics Truly Indicative of Positive Outcomes?

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Background: Patient recruitment remains a critically important and highly variable component of the clinical trial process. Published data indicate that 80% of clinical trials are delayed and approximately half of those delays are due to recruitment difficulties. Internet-related technologies for advertising (e.g., recruiting) have broadened and hastened patient recruitment in depression studies. In the current metric-driven environment, where "competitive enrollment" is the norm, expediting patient recruitment is essential. However, there is a paucity of data indicating the degree to which these e-tools and e-strategies may be impacting compliance and completion rates. Moreover, is it possible that an over-emphasis on retention and completion adds to the ever-increasing failed studies phenomenon?
Methods: We reviewed and compared the recruitment methods utilized to successfully randomize 501 depressed patients into double-blind placebo-controlled studies of investigational antidepressants at our research center. Patients in Cohort 1 (n = 286) were recruited prior to the utilization of the Internet as a mechanism for patient recruitment. The participants were in one of two large (n = 166 and n = 120) single-site positive pivotal studies. Patients in Cohort 2 (n = 215) were recruited by a combination of traditional methods, as well as vis-à-vis the Internet, encompassing nine different clinical studies. More than

half (53%) of the randomized patients were successfully recruited via Internet-based tools and strategies. Additionally, all of the patients in Cohort 2 were participants in studies where there was an extra emphasis on patient retention, "at least 70%" was the completion target.

Results: The completion rate in Cohort 1 was 45% and the non-compliance rate was 8%. An analysis of the patient drop-outs indicated that 39.7% of the patients discontinued due to a lack-of-efficacy, a.k.a. "ineffective medication." Both of the clinical trials demonstrated robust statistically significant ($p < .01$) efficacy by week 2 and at endpoint for the (then) investigational treatments. The overall completion rate in Cohort 2 was 77.2%, with 70.2% for the Internet-recruited patients and 85.1% for those recruited via the traditional methods, including television, radio and newspapers. The pooled non-compliance rate was 11.2%, with 14.5% amongst patients recruited via the Internet and 6.9% for those recruited via other methods. Perhaps most noteworthy, an analysis of patient drop-outs ($n = 49$) in this pooled Cohort 2 population indicated that only 10.2% of the patients discontinued treatment due to a lack-of-efficacy, two were recruited via the Internet and three from traditional methods. Furthermore, in the nine multi-centered studies encompassed in Cohort 2, two of the projects were categorized as "failed studies."

Discussion: Internet-based patient recruitment does not have a significant impact on patient compliance, retention and/or completion rates in antidepressant trials.

As retention and completion rates have been increasingly emphasized as key outcome measures for the past fifteen years, the incidence and prevalence of failed trials has also steadily increased, perhaps not coincidentally. The markedly decreased frequency of patients discontinuing double-blind treatment due to a lack-of-efficacy, as in Cohort 2, has been recently reported (in multi-centered studies) by several sponsors as well. We believe that an over-emphasis on retention and completion can contribute to fewer patients discontinuing participation due to a lack-of-efficacy, reduced signal detection and the increased likelihood of a "failed study."

Disclosure: C. Wilcox: Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **N. Oskooilar:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **M. Tong:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **J. Morrissey:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **D. De Francisco:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **M. Henry:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc. **D. Grosz:** Part 4: Pharmacology Research Institute receives grant funding from pharmaceutical companies to conduct clinical trials at our site(s). In calendar years 2009 to present, we have entered into Clinical Trial Agreements with Pfizer, Medivation, Nabi Biopharmaceuticals, Wyeth, AstraZeneca, Indevus, Eli Lilly, Johnson & Johnson, Forest, Bristol-Myers Squibb, Élan, Janssen Alzheimer Immunotherapy, Shire, Forest, Novartis, Takeda, Genentech, Merck, Schering-Plough and PGx Health/Clinical Data, Inc.

65. 7 Deadly Sins: Guidelines for Reporting Clinical Trials Methodology Research

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Background: Clinical trial failure rates in several disease areas are approximately 50 percent, even in trials powered at 80-90 percent. Many methodological approaches for increasing signal detection have been proposed, but variability in reporting the efficacy of these methods makes it difficult to evaluate and compare results. Standardizing reporting across studies and methodologies will alleviate these limitations and reduce reporting bias.

Methods: We propose seven requirements for statistical reporting, explain the importance of each, and where appropriate, provide a detailed illustration of how misuse or omission can influence the interpretation of study results.

Results: Report interrater reliability (IRR). IRR should be reported in all studies with multiple raters and multiple observations, although this is rarely done. Low IRR can reduce study power and the ability to detect drug-placebo separation. For severity assessments, the intraclass correlation coefficient (ICC) is required to accurately determine IRR. One common error is to treat individual items from a single severity scale as separate observations to make up for a lack of multiple complete observations. We demonstrate how ICCs calculated this way may be <i >inversely related to the reliability of a construct.

Use appropriate statistical tests. Kappa or percent agreement is often inappropriately used with continuous variables, such as using a fixed criterion (e.g., +/- 20%) to indicate rater agreement with a "gold standard" score. We demonstrate how this is highly influenced by the criterion selected and can inflate reports of IRR. Include effect size measures. Measures of effect size (e.g., Cohen's D) should be reported for all means comparisons regardless of statistical significance. Effect sizes permit readers to decide if findings are clinically relevant without regard to sample size or statistical significance. Effect size reporting allows comparisons both within and across studies with different outcome variables. Identify <i >a priori and <i >post-hoc analyses. Methodological comparisons should be identified <i >a priori in a statistical analysis plan, much like efficacy analyses. If not, they should be identified as <i >post-hoc when reported. Typically, <i >post-hoc analyses are performed on small subsets of the population. Failing

to report these details can result in over-interpretation of exploratory analyses performed on small subsets of data.
Acknowledge and correct for multiple comparisons. Authors should report all analyses if multiple comparisons are performed on a single sample, regardless of whether or not they are published. Appropriate statistical corrections must be made (e.g., Bonferroni) in order to avoid inflating false positives. Reporting a significant result on a subset of data without indicating the total number of comparisons made across the entire data set may lead to over-interpretation of false positives. This is further compounded if analyses by rater education, site enrollment levels, country, etc. are added.
Include inferential statistics for means comparisons. Statements concerning differences or patterns in means (e.g., ''numerically larger'') should be substantiated with inferential statistics (e.g., t-tests, ANOVA) which allow one to conclude if the observed difference is likely to have occurred by chance.
Correct interpretation of null hypothesis testing (NHT). NHT is commonly misinterpreted in clinical trial methodology. As Cohen (1990) notes in his classic article, common misinterpretations of NHT include concluding that smaller p values indicate more important effects, or that a non-significant p value represents a finding of no difference.
Discussion: We demonstrate how proposed guidelines for statistical reporting can standardize outcomes research on methodologies and reduce reporting bias. Empirical research evaluating the effectiveness of new methods to increase signal detection holds important consequences not only for clinical trial methodology but also for future drug development decisions facing sponsors and regulators.
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66. The Maternal and the Paternal Brain: Synchrony, Specificity, and Links with Oxytocin and Vassopresin

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Background: Mothering depends on evolutionary-based biological processes that evolved to ensure offspring survival. The expression of mothering involves specific brain structures, hormonal release, and stereotypic behaviors that combine to provide the bio-behavioral basis of motherhood. Research in human and animal models addressing the brain basis of mothering underscored the Nucleus Accumbens and the amygdale as important structures for the expression of mothering, pointed to the role of Oxytocin (OT) and Vasopressin (AVP) in maternal-infant bonding, and describes the typical behavioral repertoire associated with mothering. In contrast, nearly no study addressed the neural basis of human fathering. The current study is the first to address the coordinated neurobiology of mothering and fathering by assessing the parents' fMRI response to a dynamic, ecologically-valid infant stimuli and testing the degree to which the maternal and paternal brains synchronize in response to their own infant cues. In addition to synchrony, we examined specific functional networks associated with mothering and fathering and their associations with maternal and paternal plasma Oxytocin and Vassopressin, two neuropeptides differentially linked with female and male bonding respectively.
Methods: We assessed the BOLD fMRI response of 15 mothers and 15 fathers parents to 4-6 month old infants to video vignettes of their infant and assayed the parents' plasma OT and AVP. Mother-father whole-brain correlation analysis was used to detect specific brain areas that synchronize in mothers' and fathers' brains while viewing their own infant. Focusing on mother-father specificity, we used repeated measure GLM for mothers and fathers in which the various infant-related films were defines as distinct block predictors. Functional connectivity (FC) maps with the amygdala as seed region were computed for mothers and fathers separately, to assess gender-specific functional networks. Last, hormonal covariate analyses were computed assessing activity in specific brain areas that correlates with OT and AVP.
Results: A: Synchrony
Inter-couple correlation analysis revealed synchrony between mothers' and fathers' brains in visual cortex, pre motor and motor cortices, cerebellum, Temporal Parietal Junction (TPJ), IFG, and insula during the viewing of their own infant video. B: Specificity Gender-specific analyses revealed that mothers show higher activations then fathers in the right amygdala and the right temporal pole. Fathers show higher activations in the dPFC. Functional connectivity analysis reveals that mothers display a limbic functional network in association with the right amygdala, including the collateral amygdala and the STS. In contrast, fathers display cortical functional correlations to the right amygdala in parietal occipital and frontal gyri.
C: Links to Affiliation Neuropeptides In assessing the links between maternal and paternal OT and AVP we found that although mothers and fathers showed similar hormonal levels, different correlations emerged between the hormones and brain activity in mothers and fathers. Results show the OT is correlated to emotional limbic brain responses including in the ventral ACC, amygdala and NAcc only in mothers. In fathers OT is correlated to cognitive cortical areas such as the dmPFC. AVP is correlated only in fathers to social cognition areas such as the IFG, temporal pole and TPJ.
Discussion: Results are the first to demonstrate that mothers and fathers synchronize their brain responses to own infant in cortical areas implicated in social cognition. At the same time, findings also highlight distinct emotional networks in mothers and fathers at the limbic and cortical levels. Mothers showed greater activation at limbic-motivation areas and such activations were associated with their OT levels, suggesting a more intuitive approach to infant care. Fathers exhibited greater response in cognitive areas related to emotion modulation and mirror systems, which may suggest a more cognitive and imitation-based parental response. The synchrony found between the maternal and paternal brain and parenting-related hormones may expand current knowledge on the neurobiological basis of attachment and the roots of fathering.
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67. Capacity-Based Differences in Functional Network Activation During Spatial Working Memory

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Background: Working memory is a core cognitive function that is thought to play a role in a number of more complex, higher-level processes. However, working memory capacity varies substantially even across healthy individuals. While there are indications that white matter structure, grey-matter integrity, neural signaling changes, and other factors may contribute to this variation, the roots of these individual differences are still

under investigation. It is of particular interest to probe what neural signatures differentiate high-performing individuals, as this information may help us understand how to improve functioning in individuals who have lower performance either due to natural variation or to effects of neurocognitive disorders. Here we sought to assess differences in functional activation in a large sample of healthy individuals with a wide range of behavioral performance using functional magnetic resonance imaging (fMRI) during a spatial working memory task.

Methods: As a part of the Consortium for Neuropsychiatric Phenomics project at UCLA, we assessed 117 healthy community participants aged 21-50 years. We administered a Sternberg-style spatial working memory task with 4 levels of difficulty during fMRI. To quantify performance differences, we calculated each subject's working memory capacity using Cowan's formula. We then performed a voxel-wise analysis, corrected for age and sex, to determine which activation patterns were correlated and anti-correlated with individual working memory capacity.

Results: Across the entire group, the task elicited activation in regions previously associated with working memory, namely the superior frontal lobes, superior parietal lobes, anterior cingulate, and striatum. In addition, there was significantly decreased activation in regions associated with the default mode network, including medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), and superior temporal lobes. Notably, voxel-wise regression of working memory capacity predicting functional activation across the whole task revealed that the primary difference in activation associated with higher capacity was a more pronounced decrease in mPFC activation during task performance.

Discussion: Individuals with higher working memory capacity were characterized by more successful disengagement of areas associated with the default mode network during task performance. This effect suggests that the hallmark of high performance is dexterous coordination of interactive neural networks rather than simply increased or decreased activation in isolated task-related nodes. The finding has implications for our understanding of why certain healthy individuals have higher and lower working memory abilities. It also can inform our conceptualization of working memory deficits in patient populations, particularly those associated with neural connectivity deficits, such as schizophrenia.

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68. fMRI Evidence of Long-Lasting, rTMS-Caused Remediation of Performance Deficits in Working Memory Induced by Sleep Deprivation

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Background: While transcranial magnetic stimulation (TMS) is known to cause enhancement in various forms of cognitive processing, its effects on performance wear off within an hour, limiting its potential therapeutic use in combating cognitive deficits. However, it may be possible to take advantage of the LTP-like neuroplasticity produced by TMS to extend its effects. Two manipulations which may achieve this are 1) to functionally activate the targeted brain region by having subjects perform a relevant task during TMS application, and 2) to do so in repeated sessions. We previously reported that repetitive TMS (rTMS) improved performance in a working memory (WM) task in healthy subjects and in subjects who had undergone total sleep deprivation (SD) for 48 hours using stimulation to parietal and occipital cortex, respectively. Both stimulation targets were elements of acortical network derived from covariance analysis of pre/post SD fMRI and associated with SD-induced WM impairment. Here we attempted to prevent the development of WM impairments by stimulating while subjects performed the WM task in repeated sessions earlier in the course of sleep deprivation.

Methods: 5 Hz rTMS was applied to left lateral occipital cortex while subjects performed the WM task during four sessions over the course of 48 hours of SD, with performance assessed and fMRI recorded at the beginning and end of SD. Twenty-seven subjects, 13 receiving active rTMS (Active), and 14 Sham, completed the SD protocol. Another twenty-one (10 Active, 11 Sham) non-sleep deprived subjects were run as controls for WM performance. A covariance analysis referred to as Multivariate Linear Modeling (MLM; Worsley *et al*., 1997) was applied to the pre- and post-SD fMRI data.

Results: A significant pre/post SD fMRI pattern was extracted using MLM that completely differentiated the two groups, with the Active group activating an area directly beneath the coil location where rTMS had been applied, while the Sham group activated right parahippocampal gyrus. The Sham group exhibited typical degraded WM performance at the end of the SD period, while the Active group performed similarly to the non-SD Active and Sham controls. Importantly, the Active group showed this rTMS-induced facilitation of WM a full 18 hours after the last rTMS session. The beneficial effect was SD-specific (no differences occurred between Active and Sham control groups) and task-specific (both Active and Sham SD groups had similar degraded performance at the end of the SD period in a range of other cognitive tasks).

Discussion: Multivariate analysis of fMRI data recorded pre- and post-SD revealed a dissociation between groups receiving active and sham rTMS, and, given that only the Active group showed a change in cortex directly beneath the TMS coil, a modification in the sleep deprived brain most likely due to earlier rTMS sessions (the post-SD MRI session occurred 14 hours after the last TMS session).The fMRI results, coupled with the lack of WM SD deficit in the Active subjects but not Sham, suggest a beneficial, long-lasting neuroplastic change in WM caused by functionally-targeted rTMS applied during task performance. Thus, fMRI-guided rTMS technology was successfully used to produce alleviation of specific SD-induced deficits in a targeted cognitive task, sustained for at least an order of magnitude longer than the usual duration of cognitive improvements caused by TMS. This paradigm may prove useful in studying and improving SD-associated cognitive deficits, and cognitive deficits in general. One interesting possibility centers on the exploration of working memory function in the elderly, whose literature shares similar concepts of cognitive reserve with that of sleep deprivation. The present results suggest that the activity of the fMRI-identified SD network exhibited properties of neural reserve, a mechanism for cognitive reserve where a greater capacity or efficiency in the network allowed some individuals to maintain performance in the face of a reversible "pathology." Our results suggest that rTMS was able to enhance network activity in those who were not able to maintain performance, artificially facilitating neural reserve. While the neural mechanisms behind the rTMS-aided facilitation remain speculative, the results here demonstrate how rTMS could be applied to test the functional significance of fMRI-identified networks associated with resilience to cognitive decline.

Worsley KJ, Poline JB, Friston KJ, Evans AC (1997) Characterizing the response of PET and fMRI data using multivariate linear models. NeuroImage 6:305-319.

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69. Phasic Dopamine Transmission encodes Cached Value following State-Based Reinforcer Devaluation

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Background: Reward valuation is a fundamental psychological process that allows prioritization of behavior based on dynamic shifts in biological needs and psychological preferences.Cues are associated with the availability of rewards, provide the incentive to pursue them and guide the selection relevant behaviors (stimulus control). The mesolimbic dopamine (DA) pathway is important for mediating such processing. While it is clear that DA neurons can encode the objective value of outcomes (i.e. reward magnitude), in order for these signals to optimize choice behavior, they must also bear relevance to an animal's internal motivational state. However, the relationship between phasic DA events and changes in motivational state is not clear.
Methods: In order to investigate this issue, we trained rats deprived of both food and water (dual-deprivation) to perform a dual-reward discrimination operant task (DRD) designed to assess aspects of reward preference. Performance requires rats to operate levers beneath one of two spatially distinct light cues; illumination of one cue categorically predicts the delivery of food whereas the other predicts the delivery of water. The task consists of 60 single-reward trials and 30 choice trials. During single-reward trials, only one cue is lit followed by the presentation of both levers. Response to the lever corresponding to the lit cue yields its respective reward while response to the uncued lever yields nothing. During choice trials, both cues are lit and rats choose between levers to receive their preferred reward. We conducted fast-scan cyclic voltammetry (FSCV) during performance to assess phasic DA transmission in the nucleus accumbens core. Voltammetry sessions were carried out during dual-deprivation, specific satiety for either food or water ('satiation sessions'), and again after the reinstatement of dual-deprivation conditions. During satiation sessions, animals were given *ad libitum* access to either Noyes food pellets or water 1 hour prior to testing. Importantly, rats remained motivated for performance during all sessions by the availability of at least one valued reward.
Results: Devaluation procedures differentially affected performance on single-reward and choice trials. An immediate and robust shift in reward preference was evident for choice trials as rats selected the devalued option significantly less ($F(1,9) = 19.39$, $p = 0.002$). In contrast, presentation of cues in single-reward trials was characterized by gradual attenuation of earned rewards to devalued cues over time ($F(6,54) = 5.87$, $p = 0.001$). Similarly, voltammetric data indicated that prefeeding or predrinking gradually attenuated the magnitude of phasic DA signals to devalued cues over the course of the session ($F(6,54) = 5.87$, $p = 0.001$). Importantly, during devaluation sessions, DA signals evoked by the valued alternative cues remained unchanged from baseline. The temporal dissociation of the observed behavioral effects between choice and single-reward trials, and neurochemical consequences of specific satiety indicate that phasic DA signaling is not required for the immediate shifts in preference. Instead, the gradual attenuation of earned rewards during single-reward trials may indicate the involvement of phasic DA transmission in mediating the establishment of cached value and consequent shifts in behavior observed over the course of testing.
Discussion: During dual-deprivation sessions, the presentation of all cue types elicited robust phasic responses. The slow attenuation of phasic DA signaling elicited by devalued cues and the pattern of behavior observed during satiation sessions is consistent with the hypothesis that phasic DA transmission codes for the cached-incentive value of predictive cues. The observed systematic neurochemical and behavioral alterations may reflect the re-evaluation of cached value following changes in motivational state. If phasic DA transmission codes a cue's cached-value then diminished value would be expected to reduce that cue's capacity to drive stimulus control. It's notable, that unlike prior experiments that utilized "two-meal" pre-feeding paradigms involving the non-contingent presentation of food from behind a screen, our DRD task requires that rats process response rules and execute operant contingencies within a constrained time-frame in order to maximize their total number of preferred rewards. Thus, the DRD behavioral paradigm- when coupled with FSCV, is capable of providing a finely demarcated assessment of changes in a stimuli's subjective value and a precise assessment of the concurrent alterations in phasic DA transmission.
Disclosure: V. Martinez: None. **P. Phillips:** Part 1: Spouse is employed by Amgen., Part 2: Spouse is employed by Amgen., Part 3: Spouse is employed by Amgen.

70. The Corticotropin Releasing Factor 1 Receptor (CRF1R) Antagonist GW876008 modulates Brain Response during Extinction in Patients with Chronic Abdominal Pain, but not Healthy Control Subjects

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Background: Engagement of the central CRF/CRF1R signaling system is involved in the central coordination of the stress response, and in emotional learning. Alterations in this system have been implicated as a possible mechanism in pathophysiology of irritable bowel syndrome (IBS). Conditioned fear responses to abdominal pain and discomfort are likely to play a role in IBS symptoms. The aims of this study were to characterize the effect of the CRF1R antagonist on brain responses during acquisition and extinction of conditioned fear to an abdominal pain stimulus in age-matched female IBS patients ($n = 11$) compared to healthy controls (HC; $n = 15$) using a 2 group (IBS, HC) $\times$ 3 drug (placebo [PLA], 20 mg and 200 mg of GW876008) cross-over design and functional magnetic resonance imaging.
Methods: The fear conditioning and extinction learning protocol consisted of three phases: 1) *Acquisition* [5 trials of cue presentation (red light) followed by an aversive abdominal stimulation (electric shock to left lower abdomen)]; 2)*Test phase* [10 trials in which stimulation followed cue on 50% of the trials]; and 3) *Extinction* [5 trials presenting cue but no stimulation]. Trial duration was 15 s as was each inter-trial interval. SPM8 was used to specify a general linear model with subject as a random effect and group and drug as factors. *A priori* contrast analyses were performed to test for group differences in brain response to the cue during GW876008 compared to PLA administration in anatomically defined regions of interest for Acquisition or Extinction. Cluster and voxel-level significance was considered at $p < 0.05$ after implementing family-wise error correction.

Results: Within-group analyses indicated that even though the antagonist significantly suppressed clusters of activity in the thalamus and midbrain regions in both patients and HCs during Acquisition, no group differences were observed. In contrast, during Extinction, the CRF1 antagonist produced greater suppression of brain activity in IBS compared to HCs bilaterally for the medial prefrontal cortex (mPFC) and pons, as well as the left anterior insula (aINS), and the right hippocampus (Hipp). No regions showed greater suppression by the drug in HCs compared to IBS during Extinction. In addition, within HCs, no regions showed significant suppression during Extinction following CRF1R administration at the 200 mg dose. For IBS patients, 200 mg of the antagonist produced suppression bilaterally in the mPFC, midbrain, Hipp, thalamus, as well as the right aINS and right pons. No brain regions showed greater activation during CRF1 antagonist administration compared to PL during Acquisition or Extinction in either IBS or HC.
Discussion: CRF1 receptors appear to play a similar role in fear acquisition in both IBS patients and healthy controls. However, during extinction, CRF1 receptors play a different role in IBS most notably in regions known to modulate extinction of fear (i.e., mPFC, Hipp).
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Disclosure: J. Labus: None. **C. Hubbard:** None. **M. Fanselow:** Part 1: I have no such financial involvement., Part 2: All income is from my UCLA salary., Part 3: I have no such financial involvement., Part 4: I ahve no such grants., Part 5: No. **B. Ebrat:** None. **J. Bueller:** None. **K. Tillisch:** None. **J. Stains:** None. **G. Dukes:** None. **D. Kelleher:** None. **B. Naliboff:** None. **E. Mayer:** Part 1: Research support from GSK through University, Part 4: Research support from GSK through University.

71. Posterior Cingulate Cortex is Critical for Associative Learning

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Background: Anatomical and neurophysiological evidence suggest a role for posterior cingulate cortex (PCC) in learning and memory. PCC is situated at the intersection of brain systems involved in visual orienting, emotional and motivational processing, and memory, indicated by its connections to parietal cortex, orbitofrontal and anterior cingulate cortices, and the medial temporal lobe (MTL), respectively. Moreover, PCC hypometabolism is closely linked to early cognitive deficits in Alzheimer's Disease. Despite this evidence, the precise contributions of PCC to learning and memory remain unclear. Some models posit a role for PCC in associative learning[1], others proffer a more prominent role in valuation[2], while still others focus on a role for PCC in long-term memory[3].
Methods: To distinguish amongst these possibilities, we studied the relationship of neuronal activity in PCC to learning and memory in rhesus macaques performing a variant of the location-scene association paradigm. On each trial, monkeys saw a neutral scene photograph, then shifted gaze to one of two targets for liquid reinforcement. The correct target for each scene initially had to be learned by trial and error, and subsequently recovered from long-term memory to sustain performance. We included four scene types in a 2x2 design: highly familiar reference scenes and scenes novel for eachsession, and high and low reward size scenes. After monkeys had learned the basic task, we recorded the activity of single PCC neurons and then, in separate sessions, examined behavioral changes on this task after reversibly inactivated tissue in this region with muscimol.
Results: Monkeys learned all scene types above chance. High-value scenes were better-learned than low-value scenes, and highly familiar reference scenes were associated with very high performance. Single unit recordings revealed that firing rates of PCC neurons were modulated during the task in ways relevant to associative learning. Most PCC neurons showed a prominent error signal following incorrect choices. This error signal was larger for new scenes thanfor previously learned reference scenes. In addition, PCC firing rates tracked the value of the scene from the moment in the trial that the scene appeared. PCC firing rates over the course of the session tracked global learning rate, regardless of performance on the individual trial. Finally, muscimol injections significantly impaired learning, but performance on previously-learned scenes was unaffected. Notably, learning of high-value scenes was also unaffected during muscimol inactivation.
Discussion: These results provide strong evidence that PCC functionally contributes to associative learning but not to the storage or retrieval of information from long-term memory. This is the first study in which PCC has been reversibly inactivated in primates, and it bolsters decades of neurological, imaging, and neurophysiological evidence suggesting a role for PCC in associative learning. We conjecture that PCC serves as an adaptive controller for rate of learning.
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Disclosure: S. Heilbronner: None. **M. Platt:** None.

72. Neural Representation of Value and Prediction Error Signals in Late-Life Depression With and Without Suicidal Behavior

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Background: To commit suicide is to reject all future rewards and punishments. Could faulty estimation of these future outcomes play a role in suicidal behavior? Indeed, people with a history of suicide attempts mispredict future rewards on the Iowa Gambling Task (Jollant *et al.*, AJP, 2005; Malloy-Diniz *et al.*, JAD, 2009) and during probabilistic reversal learning (Dombrovski *et al.*, AJP, 2010). In younger suicide attempters, this misprediction has been linked to altered activity of the lateral orbitofrontal cortex (BA47) (Jollant *et al.*, NeuroImage, 2010). In this fMRI study of attempted suicide in late-life depression, we used a Bayesian reinforcement learning model to decode brain processes involved in predicting future rewards during probabilistic reversal learning.
Methods: Fifty-four participants aged 60+ (31 with major depression, 13 of whom had made past suicide attempts and 18 had no lifetime history of suicidal behavior or ideation, and 23 psychiatrically healthy controls) performed a reward-/punishment-based learning task (probabilistic reversal learning) during 3T fMRI scanning using a reverse EPI sequence. Over 300 trials, participants received occasional misleading feedback (13-20%), and experienced 12 unexpected contingency reversals. We applied a Bayesian reinforcement learning model fitted to participants' behavior (Dombrovski *et al.*, AJP, 2010) to estimate expected value and prediction error. These variables were convolved with the hemodynamic response function in first-level linear regression

models implemented in AFNI. Beta weights from these models were taken to a second-level group analysis examining effects of depression and suicide attempt history. We applied cluster thresholding to control type I error.
Results: Behavior. Both depressed groups performed worse on the task, compared to controls. Depressed participants switched their choice more, both in response to misleading negative feedback and spontaneously. We observed no effect of suicidal behavior. Model fits to behavior were better for controls than for both depressed groups. Task network. In controls, low expected value (higher uncertainty) was associated with higher BOLD signal in a frontoparietal network including the ventrolateral (BA47), dorsolateral (BA9), and dorsomedial (BA8) prefrontal, frontopolar (lateral BA10), anterior insular (anterior BA13), parietal (BA40) cortex, precuneus (BA7); regions previously implicated in cognitive control. By contrast, the ventromedial prefrontal cortex (vmPFC; medial BA10, BA32) and the posterior insula (posterior BA13) displayed higher BOLD signal in response to high expected value, as did a default network node in the posterior cingulate cortex (BA31). Positive prediction error was robustly associated with higher BOLD signal in the same "cognitive" network and in the caudate nucleus. The representation of negative prediction error was similar, but statistically weaker. Group differences. While value representations were similar in the "cognitive" frontoparietal task network across groups, suicide attempters displayed lower BOLD signal in response to high value in the vmPFC and in the posterior insula ($p < .05$, corrected), and these effects were not related to depression group status. Conversely, depression was associated with weaker positive prediction error representations in the thalamus, striatum, right insula, and superior temporal gyrus (STG; $p < .05$, corrected), and these effects were not related to history of suicide attempts.
Discussion: In this computational model-based study of depressed elderly, attempted suicide was associated with disrupted value signals in the vmPFC and posterior insula, regions that carry abstract representations of expected rewards and punishments. This disrupted expectation of future rewards in suicide attempters may be thought of as paralleling their inability to find alternative solutions in a suicidal crisis. Alternatively, our finding may be explained by increased task-irrelevant, "default-mode" processing in suicide attempters. As in previous studies of younger individuals, major depression was associated with increased behavioral reactivity to negative feedback and with blunted positive prediction error signals in the thalamus, striatum, right insula, and STG. These data give rise to a hypothesis that the tendency of depressed individuals to over-react to negative feedback may not represent merely an increased reactivity to punishment, but also result from poor encoding of previous positive prediction errors.
Disclosure: A. Dombrovski: None. **K. Szanto:** None. **L. Clark:** None. **C. Reynolds:** None. **G. Siegle:** None.

73. Neural Substrates of Real-Life Decisions to Use Marijuana: A Neuroeconomic Study

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Background: A fundamental outstanding question in substance-abuse research concerns the mechanisms of decision-making; that is, how decisions to use drugs, despite substantial negative consequences, continue to occur. Recent evidence about the neural substrates of decision-making indicates that decisions made in various contexts involve a dynamic balance between 'bottom-up', motivational processes and 'top-down' inhibitive cognitive control processes. It remains unknown what neurobehavioral systems underpin decisions to take drugs. Here, we aimed to assess the neural correlates of decisions to buy marijuana for laboratory self-administration in regular marijuana smokers, using a novel fMRI paradigm 'MJNET' (Marijuana Neuroeconomic Task), which combines fMRI with the methods of human behavioral pharmacology.
Methods: Healthy, non-treatment-seeking participants (10 males; 4 females) who smoked marijuana 6.6 (SD = 0.7) days per week sampled two doses of marijuana, 'Dose A' (5.5%THC) and 'Dose B' (0% THC), at least one week prior to participating in the MJNET session. On the morning of the MJNET session, they underwent functional Magnetic Resonance Imaging (fMRI) while completing MJNET. This task requires participants to make repeated decisions about purchasing different amounts of either Dose A or Dose B marijuana, in standardized puffs (offers range from 1 to 12 puffs of marijuana). There were three types of trial: 1) 'No Value' trials contained offers of Dose B (placebo marijuana); 2) 'Conflict' Trials were Dose A trials designed to be expensive (between $3 and $5 per puff); and 3) 'Value' trials were Dose A trials that were priced between $0.25 and $1 per puff. Participants were told prior to completing MJNET that one decision they made would be randomly selected and implemented, and that they would remain in the laboratory for 6 hours following the scan regardless of the selection. If the random selection was to smoke marijuana, the purchase price would be taken from their study payment. Thus, participants were explicitly instructed to treat each decision as if it were real, because one of them would be implemented.
Results: Data collection is ongoing. Initial data analyses indicate that, at a behavioral level, the task is functioning as intended. Participants reported wanting to smoke offers of Dose A (active) more than Dose B (placebo) marijuana [$t(12) = 4.8$, $p < 0.001$]. They also decided to purchase marijuana in a higher proportion of Value trials than No Value Trials, [$t(12) = 3.9$, $p = 0.002$], and in more Value trials than Conflict trials [$t(12) = 4.0$, $p = 0.002$]. Initial analyses of fMRI data indicated that decision-making during Value relative to Conflict trials elicited activation in brain areas implicated both in bottom-up processing (right Ventral Striatum [$z = 3.5$, $p < .001$]; left insula [$z = 3.2$, $p = .001$]) and top-down processing (right dorsolateral PreFrontal Cortex [$z = 3.1$, $p = .001$]).
Discussion: These data provide initial evidence that the methods of neuroeconomics combined with those of human behavioral pharmacology can be employed to investigate the neural substrates of real-life decisions to use drugs of abuse. Data to date indicate that decisions to use drugs such as marijuana likely involve both bottom-up and top-down processes, as has been demonstrated to be the case with other types of decision-making. Research employing MJNET is ongoing; we anticipate that use of this method will likely improve understandings of neural systems underpinning problematic drug use.
Disclosure: G. Bedi: None. **M. Haney:** None.

74. Linkages between Genetic Factors, Neurocognitive Activation, and Response to Interventions to Reduce Adolescent Alcohol Use and Related Risk Behavior

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Background: Juvenile justice-involved adolescents have substantially higher rates of alcohol abuse, alcohol-related problems, and alcohol-related risk behavior including risky sexual behavior. The development of more effective treatment requires a better understanding of the basic biological underpinnings of substance abuse and related risk behavior, about which very little is known for adolescents.

Methods: The goal of this study was to examine genetic and neurocognitive factors as they relate to alcohol-related risk and problems among juvenile justice-involved adolescents and, importantly, as they may potentially moderate response to an intervention to decrease risk behavior. Adolescents in the juvenile-justice system (age 14-18; 55% male) were randomly assigned to a group-level intervention to decrease alcohol-related risky sexual behavior (n = 94) or an information-based control intervention (n = 95). Over 80% of these youth have engaged in sexual intercourse (average age at first intercourse = 13.09) and have had an average of 6.6 sexual partners (lifetime), yet only 15.2% had used condoms 100% of the time they had had sex. Almost 20% drank alcohol once a week or more, and over 40% consumed 4 or more drinks per drinking occasion. Only 34% of participants said they "never" had sex under the influence of alcohol.
Results: In tasks assessing response inhibition (Go/NoGo) and delay of gratification, we find robust main effects consistent with prior work with both adolescents and adults. Higher genetic risk on SNPs from the CHRM2 and GABRA2 genes is associated with activation during these tasks. Importantly, we show that brain activation during the tasks significantly moderates the effectiveness of the intervention on both sexual risk (regions: IFG, DLPFC) and alcohol use (region: IFG).
Discussion: These findings indicate that genetic factors are associated with aspects of neurocognitive function that then moderate the effectiveness of behavioral intervention. Gaining a more thorough understanding of basic biological differences that underlie adolescent risk behavior and response to interventions to decrease risk will facilitate the design and targeting of risk reduction interventions for high risk adolescents.
Disclosure: A. Bryan: None. **E. Claus:** None. **R. Magnan:** None. **S. Feldstein Ewing:** None. **K. Hutchison:** None.

75. Chronic Alcohol Exposure disrupts Executive Cognitive Function and D2 receptor Modulation of Neuronal Firing in the Prefrontal Cortex of the Rat

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Background: The medial prefrontal cortex (mPFC) plays a critical role in decision-making and cognitive control of behavior, and these functions are highly influenced by dopaminergic (DA) activity in the mPFC. Previous studies in humans have reported disruption of cognitive processes following repeated episodes of excessive alcohol consumption and withdrawal, suggesting a possible link to altered mPFC function and DA activity in the mPFC. Recurrent network activity between excitatory pyramidal neurons in this brain region is shaped by recruitment of inhibitory GABAergic neurons, specifically those that are characterized as fast-spiking (FS). While these interneurons represent a relatively small population of the total number of neurons in the mPFC, they are highly interconnected and critically regulate network and synchronous activity. In addition, dopaminergic inputs to mPFC exert powerful effects on network activity via D1/D2 receptor modulation of the balance between excitatory and inhibitory tone. We propose that chronic alcohol-associated deficits in cognition may relate to alterations in dopaminergic modulation of excitatory-inhibitory tone.
Methods: To determine the effects of chronic ethanol exposure on the mPFC, we used a rat model of chronic intermittent ethanol (CIE) exposure by vapor inhalation that was followed by a 1-week period of withdrawal from exposure. Working memory performance on a variable delay non-match-to-place task was used to assess the effects of CIE exposure on PFC-dependent cognitive. Neuronal population activity in the mPFC was obtained using multielectrode recording procedures while the animals performed the behavioral task, and patch-clamp electrophysiology was used to examine D1/D2 modulation of the firing properties of pyramidal and FS-interneurons in acute slices obtained from control and CIE exposed rats.
Results: Behavioral assessment of cognitive function revealed delay-dependent impairment of working memory performance in CIE rats compared to controls. These deficits were also associated with a reduction in cognitively modulated increase in phase association of the activity of mPFC neurons to the hippocampal theta rhythm during the epoch of the task that required the rat to use working memory to guide decision-making. In acute slices obtained from both control and CIE animals, D1 stimulation (SKF38393; 5 uM) increased the firing rate of both FS-interneurons and pyramidal neurons indicating that CIE did not alter D1 receptor modulation of cortical activity. In agreement with previous reports, D2-stimulation (quinpirole, 5 uM) in slices from control animals had a differential effect on these two populations of neurons in that it decreased the firing rate of pyramidal neurons but enhanced the firing rate of FS-interneurons. However, in slices from CIE animals, D2-stimulation no longer altered the firing activity of either pyramidal neurons or FS-interneurons.
Discussion: While the cellular mechanisms that underlie the loss of D2 modulation of cortical activity following CIE are unclear and are currently under investigation, it is reasonable to suggest that this loss will significantly impact DA modulation of PFC activity and may contribute to the cognitive dysfunction associated with CIE exposure. This is consistent with observations in detoxified alcoholics of altered D2 receptor signaling. In the mPFC, D2 receptor stimulation serves to enhance inhibitory tone via recruitment of fast-spiking interneurons that in turn quiet pyramidal cell activation. This appears to enhance attentional processes through gating mechanisms and synchronization of network activity. We suggest that loss of D2 receptor modulation following CIE exposure may shift the cortical network into a D1-receptor dominated state that may contribute to cognitive deficits observed in human alcoholics.
Disclosure: H. Trantham-Davidson: None. **S. Centanni:** None. **J. Chandler:** None.

76. Extended Release Naltrexone decreases Rewarding Properties of Sucrose in Patients with Opioid Dependence

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Background: Opioid neurotransmission mediates hedonic value of sweet tastants while their intake may be exaggerated by habitual consumption of exogenous opioids (e.g., opioid dependence). Sweet Taste Test (STT) is a validated quantitative instrument assessing taste perception and hedonic features of sucrose using a randomized and double-blind administration of five different sucrose concentrations ranging from 0.05 to 0.83 Molar (Kampov-Polevoy *et al.* 1997). We hypothesized that in detoxified opioid dependent patients, administration of sustained release formulation of opioid antagonist naltrexone would modulate the hedonic and motivational processing of sucrose.
Methods: The STT and cue-induced craving task were administered to opioid dependent patients (n = 15) before and one week after the injection of extended release microcapsules containing (Depotrex®, BIOTEK, Inc, Wellesley, MA) an equivalent of 228 mg of naltrexone base.
Results: Analyses of covariance, employing sucrose concentration and its perceived taste as covariates, showed that extended release naltrexone therapy significantly reduced the self-reported hedonic ("liking", $p = 0.0001$) and motivational ("wanting", $p = 0.002$)

characteristics of sucrose. Greater reductions in both of these characteristics were associated with more diminution in the cue-induced opioid craving: $r = 0.58$; $p = 0.02$ and $r = 0.67$; $p = 0.006$, respectively.
Discussion: Opioid antagonism in opioid dependent subjects leads to a smaller sweet taste reward, which, in turn, may be proportional to decreased opioid craving. These pilot results support the heuristic value of the STT as a potential marker of response to extended release naltrexone treatment and call for further inquiry into potential clinical applications of the test.
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77. Association between Impulsivity and Risk-taking in a Sample of Adolescent-onset Marijuana Users

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Background: Prior research has indicated a sharp resurgence in adolescent substance abuse, including marijuana (MJ), starting in the early 1990's (Bachman, *et al*., 1998). To date only a limited number of studies have examined the effects of adolescent-onset MJ use on cognitive and psychosocial function. Findings from studies that have addressed this topic strongly support the notion that MJ users with earlier age of onset have more deficits in cognitive and emotional processes than users with later onset of use. Individuals with heavy MJ use have also been shown to have poorer decision-making abilities (Whitlow, 2004). Substance abuse, especially in adolescence, has been associated with reduced inhibitory function and impulsivity with researchers arguing that impulsivity is related to both initiation and continuation of substance abuse (Gullo & Dawe, 2008). The current study was completed to examine the relationship between self-reported and experimental impulsivity and executive function.
Methods: Thirty-six MJ-using individuals (age = 18.22 ± 1.48) and thirty-six healthy controls (HC) (age = 17.97 ± 2.24) participated in the current study. MJ-using participants met DSM-IV diagnostic criteria for current MJ abuse or dependence at the time of participation. Healthy controls included individuals who did not meet diagnostic criteria for current or past Axis I psychiatric disorders. MJ users and HCs completed a structured diagnostic interview (SCID or KSADS depending on participant age), a self-report measure of impulsivity, the Barratt Impulsiveness Scale Version 11 (BIS-11), and a behavioral measure of risk-taking behavior, the Balloon Analogue Risk Task (BART). The BIS is a 30 item self-report questionnaire that indexes three independent dimensions of impulsivity: Nonplanning, Motor, and Attention (Patton, *et al*., 1995). The BART is a behavioral measure of risk-taking in which participants are asked to "blow up" a computerized balloon. The participant has the option of saving the money at any point during the balloon trial and putting it in the permanent bank. If the person chooses to put the money in the permanent bank, they begin with a new balloon and the temporary bank is returned to $0. The BART outcome measure selected was Average Adjusted Pumps, which is the average number of pumps for balloons that did not explode. Participants also provided a urine sample, which were analyzed to quantify the level of nor-9-carboxy-delta 9-tetrahydrocannabinol (THCCOOH) at time of study.
Results: Significant between group differences in impulsivity were seen on the BIS subscale of Attention with MJ users reporting higher impulsivity as measured by BIS-Attention compared to HC ($p = 0.03$). No significant between-group differences were found for the BART Average Adjusted Pumps ($p = 0.15$). Interestingly, for MJ users BIS-Attention was significantly correlated with BART Average Adjusted Pumps ($r = .36$, $p = 0.03$). However, none of the BIS measures correlated with the BART measures in HCs. Neither BIS nor BART measures were correlated with cannabinoid count, age of first use, or age of regular use in MJs.
Discussion: The current study found that MJ users self-reported higher attentional impulsivity compared to HCs. MJ users endorsed feeling less focused with difficulty concentrating even when they were not under the influence of MJ. Further, their self-report of attentional impulsivity was associated with the behavioral measure of BART Average Adjusted Pumps, which is believed to be an objective measure of risk-taking. These findings suggest that in MJ users risk-taking may be associated with poor concentration during evaluation of outcomes as well as differences in perceived reward salience. Self-report and behavioral measures of impulsivity were not related to current cannabinoid count, age of first use, or age of regular use, indicating these findings are not associated with acute or chronic drug exposure. The present results have implications for understanding adolescent decision-making and neural development with respect to MJ use.
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Disclosure: E. McGlade: None. **M. Lopez-Larson:** None. **D. Yurgelun-Todd:** None.

78. Insula Functional Connectivity with Default-Mode Network is Modulated by Varenicline and Nicotine in Abstinent Smokers

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Background: The insula appears to play a critical role in nicotine addiction. Emerging evidence from intrinsic network connectivity studies also implicates insula in the modulation of dynamic activity between competitively interacting brain networks associated with internally-oriented cognitive operations (default-mode network: DMN) and externally-oriented attentional processes (executive control network: ECN). Altered network dynamics may underlie cognitive impairment and affective dysregulation often noted during smoking abstinence. Varenicline, via actions on nicotinic acetylcholine receptors, is thought to reduce smoking behaviors by ameliorating abstinence-induced effects during a quit attempt, while also dampening nicotine-induced effects during a smoking lapse. We exploit this theorized "dual action" profile to examine the impact of varenicline, both in the presence and absence of nicotine, on insula's resting-state functional connectivity (rsFC) with other brain regions. We hypothesized: 1) abstinence-induced alterations on insula-centered neural circuits would be ameliorated by both nicotine and varenicline; and 2) nicotine would have reduced impact in the presence of varenicline.

Methods: Abstinent smokers (>12 h; N = 24) completed six fMRI assessments on separate days in a randomized, double-blinded, placebo-controlled, two-drug crossover study. At three different points during a varenicline administration regime (PILL factor: pre-pill, placebo, varenicline), participants underwent imaging on 2 occasions, once each with a transdermal nicotine or placebo patch (PATCH factor). Each participant underwent varenicline and placebo pill administration for ~17 days and completed nicotine and placebo patch scans at the end of each medication period. fMRI data were collected during 8-min "resting" scans in which participants were instructed to simply relax with eyes-closed. For each smoker and session, rsFC was assessed by correlating each voxel's time course with a reference time course from a defined "seed" region. A left-mid insula seed (3024 mm‡; Talairach: -40, 6, 4) was employed that had been indentified in a previous analysis exploring amygdala-centered rsFC. The impact of varenicline and nicotine on insula's rsFC with other regions was assessed in a whole-brain PILL × PATCH interaction analysis ($p_{corrected} < 0.05$).
Results: This PILL × PATCH analysis identified several regions whose rsFC with insula was subject to pharmacological manipulations, including: ventromedial prefrontal cortex (vmPFC), dorsomedial PFC (dmPFC), posterior cingulate cortex (PCC), bilateral hippocampus, and left amygdala. Focusing on the vmPFC region of interest (ROI), rsFC with insula was greatest following abstinence and modulated by study medications in a manner consistent with varenicline's dual action profile. Specifically, nicotine (vs. placebo) decreased insula-vmPFC rsFC only in the absence of varenicline (*pre-pill*: $p = 0.003$, *placebo-pill*: $p < 0.001$; *active-pill*: $p = 0.27$). No nicotine-induced reduction was observed under active-pill conditions as varenicline: 1) acting as a *partial agonist* in nicotine's absence, was associated with a modest rsFC *decrease* (placebo-patch: $p = 0.024$); and 2) acting as an *antagonist* in nicotine's presence, was associated with a rsFC *increase* (nicotine-patch: $p < 0.001$). Similar interaction patterns were observed for all ROIs. Additionally, to investigate the relationship between nicotine withdrawal and insula's rsFC strength with other regions, an exploratory whole-brain correlation analysis was performed using self-reported withdrawal symptoms from each smoker and session to predict the corresponding session's connectivity values. Greater rsFC between insula and vmPFC, dmPFC, and PCC covaried with increased withdrawal in regions overlapping those showing drug-induced effects.
Discussion: Varenicline and nicotine *decreased* abstinence-induced alterations in rsFC between insula and constituent regions of the canonical DMN. These data support proposals derived from recent intrinsic network connectivity research that insula influences moment-to-moment information processing by toggling activity between large scale brain networks. During nicotine withdrawal, the insula, serving an interoceptive monitoring role, may bias processing towards the DMN network and away from the ECN, a shift that may underlie objectively assessed cognitive impairments during abstinence.
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Disclosure: M. Sutherland: None. **A. Carroll:** None. **B. Salmeron:** None. **T. Ross:** None. **E. Stein:** None.

79. Molecular Genetic Evidence for a Psychosis Phenotype

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Background: The last decade has seen an exponential growth in our understanding of the molecular genetics of psychiatric illnesses such as schizophrenia, schizoaffective disorder and affective disorders. Despite considerable evidence of overlapping genetic risk factors for these illnesses, however, little attention has been directed towards identifying genetic variants that may confer risk for symptom dimensions that may be shared amongst the diagnostic groups studied. For example, considerable evidence suggests that *NRG1* confers risk for several major Axis I disorders but few studies have systematically examined the effects of genetic variation at this loci on symptom dimensions that traverse diagnostic boundaries. Moreover, several reports have identified symptom-specific genetic associations within individual diagnostic groups but there are a paucity of data seeking to assess these associations in other diagnostic groups. For example, several reports have linked variation in *KCNN3* to negative symptoms in schizophrenia but there are no data seeking to assess this relationship in bipolar disorder. Data seeking to elucidate relationships between previously identified risk variants and phenotypic characteristics across diagnostic boundaries may provide novel insights into the pathophysiology of psychosis.
Methods: We genotyped a multi-diagnostic group of 764 Caucasian patients with psychosis and 193 healthy individuals using a custom Golden Gate Illumina 1536 SNP chip. The chip was designed to comprehensively assess several genes that showed prior association to multiple diagnostic groups (*NRG1, ERBB3, CACNA1C, ZNF804A* and *ANK3)* as well as several genes that showed prior association to symptom domains within specific diagnostic groups (*CCKAR, DRD4, ORC3L, SLC6A4, KCNN3, RGS4, SLC6A3* and*BAI3*). Using lifetime ratings of psychosis we then assessed the relationship between genetic variation at these loci and phenotypic variation without regard to diagnostic group membership.
Results: After correction for multiple testing, we replicated and extended findings linking 1) *CCKAR, DRD4* and*ORC3L* to positive symptoms; 2) *KCNN3* to negative symptoms; 3) *BAI3* and disorganized symptoms. Moreover, we found evidence for multiple effects of *CACNA1C, ERBB3* and *NRG1* on phenotypes shared amongst the diagnostic groups. With the exception of one SNP in ERBB3, none of these variants showed an association to any of the diagnostic groups.
Discussion: These data provide support for the utility of studying the role of genetic risk variants on a broad psychosis phenotype. The present results suggest that delineation of the role of specific genes on symptom dimensions, rather than diagnostic entities may represent a more refined approach to understanding the etiology of psychiatric disorders characterized by psychosis and may suggest novel treatment targets for specific dimensions of illness.
Disclosure: P. DeRosse: None. **C. Morell:** None. **T. Lencz:** Part 1: Eli Lilly. **A. Malhotra:** Part 1: Eli Lilly Merck Sunovion Pharmaceuticals Inc. Shire Genomind PGx Health, Part 2: Sunovion Pharmaceuticals Inc. Genomind, Part 4: Eli Lilly.

80. Unemployment and Substance Misuse and Disorder in the United States during Economic Recession

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Background: Economic stress can be both a cause and an outcome of substance misuse/disorders. Furthermore, as the economic situation changes, the link to substance misuse/disorders may also change. This presentation addresses the relationship of unemployment to substance misuse/disorders: 1) Is unemployment related to substance misuse/disorders? 2) Does the relationship change in economic recession? 3) Does the relationship vary by age, race/ethnicity, sex or USA geographic region?
Methods: Data are from the 2002 through 2009 Substance Abuse and Mental Health Services Administration (SAMHSA) sponsored National Survey on Drug Use and Health, examining the civilian,

non-institutionalized USA population (N > 450,000 across the eight included years).
Results: In 2009, among ages 18 and older, unemployment was significantly related to substance misuse/disorders (illicit drug use, heavy alcohol use, tobacco use and alcohol/drug disorders). For example, past month illicit drug use was prevalent among 8.0% in full time employed, 11.5% in part-time employed and 17.0% in unemployed. Further, although the numbers of unemployed increased markedly in 2009, the relationship of unemployment to substance misuse and disorders remained generally consistent across 2002 to 2009 and among sex, race/ethnicity, geographic region and age subgroups (except that alcohol misuse and disorders were not more common in the 18 to 25 year unemployed group).
Discussion: With an economic recession, the number of unemployed individuals misusing substances markedly increased; yet, the rate of substance misuse/disorders among the unemployed remained generally stable. Thus, the association of employment status with illicit drug use remains highly significant and persists during economic recession.
Disclosure: W. Compton: Part 1: Pfizer, Abbott Laboratories, Part 2: None, Part 3: None, Part 4: None, Part 5: N/A. **J. Gfroerer:** None. **K. Conway:** None.

81. Item Response Theory Analysis of DSM-IV Amphetamine Use Disorder Criteria in an American Indian Community Sample

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Background: One important approach to examining whether DSM symptoms of a disorder represent one or more diagnostic constructs, and whether they represent the full range of potential symptom severity, is the use of Item Response Theory (IRT). IRT is a technique for evaluating the psychometric properties of assessment criteria or "items." IRT, like classical test theory, posits that an unobserved latent construct or trait can be measured by a group of items. However, IRT provides superior results to classical test theory in having more theoretically justifiable measurement principles as well as advantages for understanding item behavior. IRT analysis provides information as to where on the latent trait dimension an item has a 50% probability of endorsement and how rapidly an item's probability of endorsement changes across the latent trait dimension. When applied to substance use disorder diagnostic criteria, IRT analysis uses the diagnostic criteria as items and generates a latent trait dimension which can be considered the underlying severity of the substance use disorder. IRT analysis of AUD DSM-IV diagnostic criteria in a Native American sample has not been previously reported. This study sought to apply IRT analysis to data obtained from a sample of 310 Southwest California (SWC) Indian participants living on contiguous reservations who had used amphetamine 11 times.
Methods: Demographic information, amphetamine use history, and DSM-IV amphetamine abuse (AA) and amphetamine dependence (AD) criteria were obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). A two parameter IRT model was generated using the BILOG-MG statistical program. The model yielded marginal maximum likelihood estimates of discriminant (*a*) and threshold (*b*) parameters for each AA and AD criterion.
Results: The 11 DSM-IV AA and AD criteria [*a* (S.E.), *b* (S.E.)] ranged from least severe, the AA criterion of role failure [0.93 (0.15), -1.18 (0.16)], to most severe, the AA criterion of legal problems [0.73 (0.13), 1.05 (0.18)]. All AD criteria were intermediate in severity between these two AA criteria and occupied the moderate portion of the severity continuum.
Discussion: IRT analysis of the NESARC dataset, a U.S. nationally representative sample, has shown that DSM-IV AA and AD criteria occur along a single continuum of severity. In that analysis, AA and AD criteria were intermixed in terms of severity and occurred in the moderate and severe portions of the continuum. The exception was legal problems, which occurred in the very severe portion of the continuum. These findings suggest that AA and AD would be better conceptualized as one disorder, AUD, as is currently being considered for DSM-V. Similar to the findings from the NESARC sample, the findings of this study of an American Indian community sample suggest that DSM-IV AA and AD are not distinct disorders, but instead measure the same underlying disorder, best conceptualized as AUD. Moreover, the current DSM-IV AA and AD criteria do not measure the mild portion of the underlying AUD severity continuum, suggesting that new criteria assessing that portion would be useful. In contrast to the NESARC sample, AA and AD criteria showed a different order of severity and occupied, as a group, a less severe portion of the underlying AUD severity continuum. These differences probably arise from differences in the samples, including, importantly, that this American Indian sample was conditioned on 11 times use as opposed to 1 times use in the NESARC sample analysis. Other differences in samples, including differences in sample size as well as unanalyzed environmental and/or biological differences, may account for these differences in results of the IRT analyses. Careful identification of symptoms which indicate more severity on the AUD latent trait severity continuum in samples from different ethnicities may be helpful in screening and brief intervention in the clinical setting and in identifying subsets of individuals with varying genetic and/or environmental risk for research studies (supported by AA10201, DA030976).
Disclosure: D. Gilder: None. **C. Ehlers:** None.

82. Risk Factors for Illicit anabolic-Androgenic Steroid Use in Men: Results from a Cross-Sectional Cohort Study

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Background: Some two million men in the United States, and millions more worldwide, have abused anabolic-androgenic steroids (AAS) to gain muscle and lose body fat. AAS abuse appears to be associated with a range of adverse consequences, including particularly cardiovascular toxicity, suppression of normal male neuroendocrine function, major mood syndromes associated with both AAS exposure and AAS withdrawal, and a substantial risk of developing an AAS dependence syndrome. Thus, given the prevalence and potential consequences of AAS abuse, it represents a major public health problem both in the United States and in many other Western countries, including especially British Commonwealth countries and Scandinavia. However, the psychosocial antecedents of AAS abuse remain arguably the least understood of all major forms of drug abuse. It is well-established that virtually all AAS abusers are male and lift weights, so that these attributes are effectively a prerequisite for becoming an AAS abuser. However, *within* the population of men who become weightlifters, the risk factors for subsequent AAS use remain poorly understood.
Methods: We evaluated 233 experienced male weightlifters, recruited from gymnasiums in three regions in the United States (Boston, Massachusetts; Palm Beach, Florida; and Los Angeles, California). Men were recruited for a "study evaluation" without disclosing the investigator's focus on AAS use. Of the men evaluated, 102 (44%) reported lifetime AAS use and 131 (56%) reported no history of AAS use. We used measurements of bodily fat-free mass index, plus urine and hair testing for drugs of abuse, to help to ensure that the study participants were being truthful about the AAS and other drug-use histories. Using a variety of verbal interviews and computerized questionnaires, we then

assessed participants' childhood and adolescent attributes retrospectively using structured clinical interviews and computerized questionnaires. This "*cross-sectional cohort*" approach—a design that we have formally presented in the recent methodological literature (Hudson JI, Pope HG Jr, Glynn RJ. *Epidemiology* 2005;16:355-9)—utilizes a study cohort, not selected for outcomes of interest, wherein exposures and outcomes are assessed retrospectively. We hypothesized that *conduct disorder* and *body-image concerns* would be major risk factors for subsequent AAS use among male weightlifters.
Results: Within the study population, many childhood and adolescent attributes showed little association with AAS use, but conduct disorder and body-image concerns showed strong associations. For individuals with prior conduct disorder vs. those without, the hazard ratio [95% confidence interval] for subsequent AAS use was 2.2 [1.5, 3.4]. For individuals in the middle vs. lowest tertile of scores on a retrospective adolescent "muscle-dysmorphia" scale, the hazard ratio was 1.5 [0.84, 2.6]; for the highest vs. lowest tertile, the hazard ratio was 3.3 [2.0, 5.3]; and for the linear trend of hazard ratios, $P < 0.001$.
Discussion: Conduct disorder and body-image concerns represent important risk factors for AAS use among male weightlifters. These observations are particularly striking when it is considered that they were obtained within a population of male weightlifters, since body-image concerns (and possibly also conduct disorder) are likely also risk factors for weightlifting in the first place. Thus the study may have substantially underestimated the *total* contribution of these risk factors to AAS use. Assessment of these attributes among young men may help to identify those who are most likely to require interventions to discourage this form of substance abuse.
Disclosure: J. Hudson: Part 1: Alkermes, Eli Lilly, Ortho-McNeil Janssen Scientific Affairs, Otsuka, Pfizer, Roche, Shire, Part 2: Pfizer, Part 3: None, Part 4: Eli Lilly, Ortho-McNeil Janssen Scientific Affairs, Otksuka, Shire. **G. Kanayama:** None. **H. Pope Jr:** Part 1: Alkermes, Part 2: None, Part 3: None, Part 4: Solvay.

83. Nonmedical Prescription Opioid Use and Use Disorders Secondary to Nonmedical Use among U.S. Young Adults by Educational Attainment

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Background: The nonmedical use of prescription opioids (NMUPO) is second only to marijuana as the most prevalent form of illegal drug use among young adults. Most of the studies among young adults have focused solely on college student samples, in which minority young adults are usually under-represented compared to non-Hispanic Whites (47% of non-Hispanic Whites, 34% of non-Hispanic Blacks, 29% of Hispanics, 61% of Asians, and 24% of Native-Americans). Less is known about prescription opioid use and consequences among young adults not pursuing higher education. During 2005-9 in the U.S., only 38-41% of 18-24 year olds attended college. The goal of this study is to better understand NMUPO and prescription opioid use disorder among 18-22 year olds who are not receiving school-based educational and preventive messages. The aims are 1) to explore whether there are prevalence differences in NMUPO and prescription opioid use disorder among young adults with different educational standing, testing for socio-economic (SES), gender and racial/ethnic differences; and 2) to investigate educational disparities in the association of psychiatric comorbidity (past-year serious psychological distress) with NMUPO and prescription opioid disorder.
Methods: We analyzed data from 24,557 young adults aged 18-22 from the 2008 and 2009 National Survey on Drug Use and Health public use files. Outcomes were: lifetime and past-year NMUPO and past-year prescription opioid use disorder (abuse/dependence) due to nonmedical use. Independent variables were: current educational attainment (current college student, high school graduate/GED [general education certification], did not complete high school), race/ethnicity, gender, annual family income, insurance status, type of county and past-year psychological distress. Data were weighted to reflect the complex design of the NSDUH sample and were analyzed by Stata 11.0 software. Basic contingency tables were followed by weighted bivariate and multivariate logistic regression models. Taylor series estimation methods (STATA 'svy' commands) were used to obtain proper standard error estimates.
Results: The prevalence of lifetime and past-year nonmedical use of prescription opioids was higher among young adults with less educational attainment (less than a high school education- lifetime: 25.3%, past-year: 13.4%; high school diploma /GED- lifetime: 25.4%, past-year: 13.0%) as compared to those attending college (lifetime: 21.2%, past-year: 11.6%, $p < 0.05$). Similar to college students, males, and those with past-year serious psychological distress not attending college were more likely to be lifetime and past-year NMUPO. Similar to college students, Native-Americans and those with more than one race were as likely as non-Hispanic Whites to be lifetime and past-year NMUPO, both groups higher than non-Hispanic Blacks and Hispanics. Different from college students, among those with less than high school education, the prevalence of lifetime NMUPO was higher among those with lower annual family income and those without health insurance. Among past-year users, those with lower educational attainment (less than high school education: 19.9%, OR: 2.03[1.43-2.88], completed high school/ GED: 16.7%, OR:1. 63[1.20-2.24]) were more likely to have a past-year prescription opioid use disorder as compared to college students (10.9%).There were no gender differences in the likelihood of having a past-year prescription opioid disorder among college students and those with less than high school education, but females with a high school diploma /GED were less likely than males with the same educational attainment to have a past-year disorder. Among users, Hispanics, Native-Americans and Asians were as likely as non-Hispanic Whites in either educational group to have past-year prescription opioid disorder. Among users, non-Hispanic Blacks (15.7%) with a high school diploma /GED were as likely as non-Hispanic Whites (18.0%, $p = 0.883$)with the same educational attainment to have past-year prescription opioid disorder. Similar to college students, among those with lower educational attainment, NMUPO with past-year serious psychological distress were three times more likely to have a past-year prescription opioid disorder as compared to those with similar educational attainment and without psychological distress.
Discussion: Prevention programs to reduce nonmedical use of prescription opioids and disorders are also needed for non-college attending young adults. Young adults with low family income and serious psychological distress appear to be especially vulnerable to consequences of nonmedical use of prescription opioids.
Disclosure: S. Martins: None. **L. Chen:** None. **M. Fenton:** None. **K. Keyes:** None. **C. Storr:** None.

84. Cocaine Dependence specifically predicts Historical Suicide Attempts among African-American Participants in a Community Corrections Program

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Background: Rates of death by suicide and lifetime suicide attempts are lower in African-Americans than in U.S. whites, both in the general and criminal justice populations. However, the

lifetime prevalence of suicide attempts among blacks in the United has risen to 4.1. Different risk factors predict death by suicide and suicidal behavior between African-Americans and White Americans, weighting substance use, particularly alcohol and cocaine use, and schizophrenia more than depression. Suicidal behaviors have 3 to 10 fold higher prevalence in criminal justice populations than the general population and any history of criminal justice involvement has recently been linked to a 2 3 fold higher suicide risk. Few studies have examined suicidal behavior among participants in community corrections programs, who carry multiple suicide risk factors including substance use disorders and history of trauma and psychiatric disorders.

This study sought to identify factors correlated with historical suicide attempts and ideation among black men, black women, white men and white women in a community corrections population largely composed of drug related felonies.

Methods: Self-report data was analyzed from 18,753 enrollees in community corrections. Characteristics of the three groups: ideators only, attempters, and controls were compared using univariate procedures. Logistic regression analyses were conducted to determine associations between historical suicidal ideation and attempts among four demographic groups: black men, black women, white men and white women.

Results: The overall prevalence of lifetime suicide attempts in this community corrections population was 7.7%, with 63% of those with lifetime suicidal ideation also endorsing prior suicide attempts. Participants with historical suicide attempts tended to be younger, white, female, take psychiatic medication, have a history of physical or sexual abuse and meet criteria for dependence on alcohol, amphetamines, cocaine, opioids, or sedatives. After multinomial logistic regression was performed, only two variables predicted suicide ideation only across all four race/gender groupings: taking psychiatric medication, and history of sexual or physical abuse. These two variables also predicted suicide attempts for all four race/gender groups, along with younger age, being on disability or retirement, and cocaine dependence. For white men and women, dependence on alcohol, amphetamines, cocaine, or opioids predicted suicide attempt and sedative dependence predicted attempts in white women only. For black women only marijuna and cocaine dependence and for black men only cocaine dependence predicted suicide attempts.

Discussion: Community corrections participants had high rates of historical suicide attempts with unique correlates differentiating attempters from ideators only among different racial and gender groups. Cocaine dependence stands out as a universal predictor of suicide attempts, while other substance dependencies show specific racial and gender profiles. Cocaine dependence was the most prevalent drug of abuse in this population, with over a quarter of the total population and half of those with a suicide attempt meeting criteria for Cocaine Dependence. Only Cocaine Dependence predicted attempts in black men. Cocaine more than other drugs may increase suicidal attempts by increasing impulsivity by creating difficulty shifting attention and problems with inhibitory control. Our findings are consistent with Nock,Hwang, Sampson, and Kessler (2010) who suggest that mental illnesses such as depression may lead to suicidal ideation whereas factors that contribute to anxiety or impulsivity move from suicidal ideation to an attempt.. Further, substance abuse and domestic violence may have additive effects on risk of suicide attempts, particularly in African-American women

This study provided a comprehensive examination of suicidal behavior by race and gender group in a community corrections population enriched for suicide risk factors, but residing in the community with access to lethal means. The supervision requirements for this population provide an excellent mechanism to screen and intervene early with high risk populations. The differences in risk factors for suicide attempts outlined in this study suggest starting points for intervention, particularly treatment of cocaine dependence in the African-American community corrections population.

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85. Childhood Emotional Abuse and Alcohol Dependence in Adulthood: Mediating Effects of Neurotic Personality Traits and Anxiety Symptoms Among Treatment-Seeking Alcohol Dependent Individuals

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Background: Alcohol dependence has been linked to a history of depression and anxiety, as well as to increased levels of the personality domain of neuroticism. These risk factors have, in turn, been linked to childhood adversity, which in and of itself is a risk factor for developing alcohol dependence. We hypothesize that the relationship between childhood adversity and alcohol dependence may be due, in part, to the mediating effects of personality and affective mood. In this study, we attempt to test this hypothesis using multiple mediation analysis.

Methods: Data were obtained from 267 alcohol dependent subjects (92 females, 175 males) undergoing inpatient detoxification and treatment in the NIAAA research program. A sample of 102 healthy control subjects (34 females, 68 males) were also assessed for adverse childhood experiences (ACE) as a comparison group. ACE were assessed in both groups using the Childhood Trauma Questionnaire (CTQ). Alcohol dependent subjects were further assessed using the Alcohol Dependence Scale (ADS) for alcohol dependence severity, the Addiction Severity Index (ASI) for lifetime alcohol-related problems, the revised NEO personality inventory, and the Comprehensive Psychopathological Rating Scale (CPRS) for depression and anxiety symptom ratings during inpatient detoxification and treatment. Multiple mediation analyses were conducted for the alcohol dependent subjects using each abuse/neglect category measured by the CTQ (emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect) individually as independent variables.

Results: Adverse childhood experiences were highly prevalent in alcohol dependent subjects. Compared to healthy controls, alcohol dependent subjects were more likely to have experienced childhood emotional abuse [odds ratio (OR) = 7.0, 95% C.I. = 3.7, 13.1], physical abuse (OR = 4.0, 95% C.I. = 2.3, 6.9), sexual abuse (OR = 4.1, 95% C.I. = 2.0, 8.5), emotional neglect (OR = 3.1, 95% C.I. = 1.9, 5.2), and physical neglect (OR = 2.1, 95% C.I. = 1.2, 3.7). Results of the multiple mediation analyses indicated that emotional abuse, when controlling for the other abuse/neglect categories, had a significant direct effect on alcohol dependence severity ($B = 0.34$, $p = 0.03$), as well as significant indirect effects through anxiety symptom ratings (bootstrap estimate of $B = 0.09$, 95% C.I. = 0.004, 0.24) and neuroticism (bootstrap estimate of $B = 0.15$, 95% C.I. = 0.05, 0.27). Additional analysis using the sub-facets of neuroticism indicated indirect effects of emotional abuse on alcohol dependence severity through the sub-facets of depression (bootstrap estimate of $B = 0.11$, 95% C.I. = 0.007, 0.26) and impulsiveness (bootstrap estimate of $B = 0.09$, 95% C.I. = 0.02, 0.22).

Discussion: Our findings are consistent with previous studies that indicate childhood adversity is a risk factor for alcohol dependence. The pathway by which childhood adversity ultimately influences the development of alcohol dependence in undoubtedly complex. Our data suggest that this pathway, at least in part, involves depressive and impulsive personality traits, and elevated

levels of anxiety symptoms during acute detoxification. Future analyses will address the potential contribution of genetic factors to these relationships.
Disclosure: M. Schwandt: None. **D. Hommer:** None. **M. Heilig:** None. **T. George:** None. **V. Ramchandani:** None.

86. Whole Transcriptome and Methylome Sequencing in the Alcohol Post-dependent Rat Model

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Background: The aim of this study is to investigate mechanisms underlying the long-term neuroadaptations induced by repeated cycles of alcohol intoxication and withdrawal. Recent data suggest that epigenetic alterations, which exert lasting control over gene expression without altering the genetic code, may play a role in several diseases including drug addiction. We hypothesized that DNA methylation may be involved in the neuroadaptations induced by prolonged alcohol exposure. Using next generation sequencing, we measured whole transcriptome and methylome in the medial prefrontal cortex (mPFC) to analyze the global regulation patterns associated with alcohol dependence.
Methods: Rats were exposed for 7 weeks (14hour/day) in vapor alcohol chambers (Blood Alcohol Concentrations $\sim$ 150-250 mg/dl). Total RNA and DNA were isolated from the mPFC using the RNA/DNA/protein purification kit from Norgen Biotek corp®. Total RNA was run through the whole transcriptome sequencing protocol (n = 4/group). The methylated DNA fragments were captured with MBD2b/MBD3L1 heterodimerand sequenced following the DNA Chip sequencing protocol (Illumina®). Log2 transformation and normalization of the raw data using the Limma package from Bioconductor was used. WT and DNA Chip sequence reads were mapped to rat genomic sequences (UCSC rn4) using Bowtie.
Results: Whole transcriptome and DNA Chip sequencing generated a total of 3.4 million and 7 million reads, respectively. 566 genes were found to be differentially regulated within our data set. 318 of the 566 genes were significantly down regulated. Ingenuity® analysis shows that most of the down regulated genes are involved in synaptic plasticity (synapsin I, syntaxin IA, DRD2). DNA methylation, induced by chronic alcohol exposure, may account for the significant decrease in gene expression in the mPFC.Furthermore, in accordance with this hypothesis, we found that increased methylation in the brain by injection of L-methionine (0.5g/kg, i.p) increases ethanol self-administration, similar to what is seen in our post-dependent rat model.
Discussion: Our results show a significant alteration of mRNA expression in the mPFC following a history of alcohol dependence. DNA Chip sequencing data currently being analyzed will help us better understand the effect DNA methylation has on gene expression regulation induced by alcohol exposure.
Disclosure: E. Barbier: None. **J. Tapocik:** None. **J. Schank:** None. **K. Schuebel:** None. **Z. Zhou:** None. **Q. Yuan:** None. **D. Goldman:** None. **M. Heilig:** None.

87. The Involvement of Alpha3-Containing Nicotinic Acetylcholine Receptors in the Habenulo-Interpeduncular Pathway in Nicotine Self-Administration

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Background: Allelic variation in the *CHRNA3-CHRNA5-CHRNAB4* gene cluster, which encode the alpha3, alpha5 and beta4 nicotinic acetylcholine receptor (nAChR) subunits, has been repeatedly associated with a significantly increased risk of tobacco addiction in humans. Until recently, very little research has focused on the role of these nAChR subunits in the motivational properties of nicotine, the major addictive agent in tobacco smoke. We recently demonstrated that alpha5-containing nAChRs in the medial habenulo-interpeduncular (MHb-IPN) pathway exert an inhibitory influence on nicotine intake, particularly when high doses of the drug are available for consumption. Similar to the alpha5 nAChR subunit, the alpha3 nAChR subunit demonstrates a restricted distribution pattern in the brain, with particularly dense expression in MHb-IPN pathway. Here, we investigated the role of alpha3 nAChR subunits in regulating nicotine consumption in rats.
Methods: A lentiviral vector was developed to selectively knockdown expression of the alpha3 nAChR subunit gene (Lenti-CHRNA3). Rats were injected with the Lenti-CHRNA3 or empty vector (Lenti-control) stereotaxically into either the MHb or IPN. Thereafter, they were trained to respond for food reward under a fixed ratio 5, time-out 20 sec (FR5TO20 sec) schedule of reinforcement during 1-h daily testing sessions. Next, the rats were permitted access to a training dose of nicotine (0.03 mg kg^{-1} per infusion) for intravenous self-administration. After stable responding on the training dose, a full nicotine dose-response function was characterized.
Results: Rats injected with the Lenti-CHRNA3 and Lenti-Control vectors into the MHb or IPN similarly responded for food reward and acquired nicotine self-administration behavior at the lower training nicotine dose. However, when provided access to a range of doses, rats with selective knockdown of alpha3 nAChR subunits in either the MHb or IPN persisted in responding for nicotine infusions at higher levels than control rats at higher unit doses of the drug.
Discussion: These data demonstrate that alpha3-containng nAChRs in the MHb and IPN exert an inhibitory influence on nicotine intake similar to that previously reported for alpha5-containing nAChRs and suggest that alpha3 and alpha5 nAChR subunits may combine to form a functional receptor subtype in the MHb-IPN tract that negatively regulates nicotine intake. Together, these findings reveal fundamental insights into the mechanisms of nicotine reinforcement that are likely to have clinical and therapeutic implications.
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Disclosure: C. Fowler: None. **Q. Lu:** None. **P. Kenny:** None.

88. Studies Performed in Rodents and Primates Point to a Potential Role for *FAM111A* in the Etiology of Alcohol Use Disorders

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Background: A variety of animal species have been used to model certain aspects of alcohol use and addiction. Strains of rats that have been selectively bred for alcohol preference have been enormously informative for identifying relevant intermediate phenotypes and for determining genetic contributions to alcohol consumption and related traits, and studies performed in macaques have repeatedly demonstrated their value for identifying genotype-phenotype relationships that translate to the human condition. In this study, we performed RNA-Seq in brain for two selectively bred lines of rats (P and NP) to identify differentially expressed genes. We also performed ChIP and RNA-SEQ in brain tissue from rhesus macaques to identify putative functional variation, followed by a confirmatory candidate-gene based study for alcohol consumption in the rhesus macaque.

Methods: We conducted a strand-specific RNA-Seq to examine genome-wide gene expression in the hippocampus of the alcohol-preferring (P) and non-preferring (NP) rat lines, and ChIP and RNA seq was performed using archived rhesus macaque hippocampus to identify genetic variation. For genotype-phenotype analyses in the macaque, animals were tested for four weeks for consumption of an 8.4% alcohol solution using a modified two-bottle free choice paradigm, and effects of genotype on alcohol consumption was analyzed using ANOVA.
Results: Among the 485 differentially expressed (FDR < 0.05) genes for the P and NP rat lines, the Fam111a gene showed an over twenty-fold difference between the P and NP lines. The higher expression of Fam111a in the P line was confirmed by quantitative RT-PCR. 13 SNPs were discovered in the rhesus macaque FAM111A gene, two of which were nonsynonymous (Chr14:14659752 and Chr14: 14660083) and one of which was present near the transcription start site, in a region rich with transcription factors binding sites and within the macaque H3K4me3 binding peak. The promoter SNP (Chr14: 14668257) was predicted to disrupt GR, BDN4 and AP-1 binding sites. The nonsynonymous SNPs did not significantly affect alcohol consumption ($P = 0.7$ and $P = 0.3$, respectively), but animals carrying SNP 14668257 exhibited alcohol consumption levels that were 2.5 times higher than those observed in animals homozygous for the ancestral allele (GG vs. GC, $F(1, 66) = 30.14$, $P < 0.0001$).
Discussion: This study used varied approaches across animal species that have been established to be useful for modeling risk for human alcohol use disorders. RNA-seq performed in the P and NP rat lines showed that the Fam111a gene was differentially expressed in brain, and a genotype-phenotype association analysis in the macaque using putatively functional variants confirmed that FAM111A genotype contributes to individual differences in patterns in alcohol use. The FAM111a gene encodes a serine-endopeptidase, but little is known about its exact function. Despite this, it has been shown that rats bred for a model of depression (Flinders Sensitive Line) exhibit increased expression levels for FAM111A RNA in brain compared to resistant lines (Flinders Resistant Line), and studies in humans show links between peripheral FAM111A expression levels and nicotine dependence. These data suggest that the FAM111A gene may contribute to risk for affective and addictive disorders in humans.
Disclosure: S. Lindell: None. **Z. Zhou:** None. **Q. Yuan:** None. **M. Kimura:** None. **J. Higley:** None. **D. Goldman:** None. **C. Barr:** None.

89. Role of the Alpha-2 Nicotinic Acetylcholine Receptor Subunit in Nicotine Reinforcement and Withdrawal

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Background: Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand gated ion channels comprised of homomeric alpha ($\alpha7$, $\alpha9$) or heteromeric alpha ($\alpha2$-7, $\alpha9$, $\alpha10$) and beta ($\beta2$-4) subunits. Genome wide associations studies in humans have found polymorphisms in a number of nAChR genes, which are correlated with tobacco addiction as well as related disorders, such as lung cancer. Significant evidence highlights the *Chrna5 – Chrna3 – Chrnb4* gene cluster and its association with tobacco addiction, while additional candidate gene studies have suggested a role for polymorphisms in the $\alpha2$ nAChR (*Chrna2*) subunit gene with tobacco addiction as well as general drug dependence. Using a *Chrna2* null mutant mouse, we asked if $\alpha2$*-containing nAChRs play a role in nicotine reinforcement and withdrawal behaviors.
Methods: For nicotine reinforcement experiments, mice were trained to lever press for food using an escalating schedule of reinforcement (fixed ratio, FR 1 to 5). Subsequently, mice were tested for intravenous nicotine (0.03 mg/kg/infusion) self-administration at a FR5 schedule for one week and evaluated on a nicotine dose response curve (0, 0.01, 0.03, 0.1, 0.4 mg/kg/infusion). For nicotine withdrawal studies, mice were administered nicotine for two weeks at 24 mg/kg/day via an Alzet osmotic minipump. On the 13th day of nicotine exposure, animals were placed into a novel environment, habituated for 40 min, administered mecamylamine (3 mg/kg, i.p.) and evaluated for somatic symptoms of withdrawal for an additional 20 min. Two hours post-withdrawal assessment, animals were sacrificed, the interpeduncular nucleus (IPN) dissected (a primary brain region expressing $\alpha2$ nAChR transcripts), and neurotransmitter levels quantified using high performance liquid chromatography coupled with electrochemical detection.
Results: For nicotine reinforcement experiments, there was no significant difference between wild-type and *Chrna2* null mutant mice during either food or nicotine self-administration. For nicotine withdrawal experiments, when quantifying somatic withdrawal signs (paw tremors, head-shakes, backing and curls) our results showed that both wild-type and *Chrna2* null mutant mice exhibit significant enhancement (versus saline) of mecamylamine precipitated somatic withdrawal scores. When we quantified other somatic behaviors (grooming, scratching, chewing, cage scratching, head nodding, and jumping), we observed suppression of behavior in wild-type mice, which failed to reach statistical significance in the *Chrna2* null mutant mice. The combined findings suggest that $\alpha2$*-containing nAChRs do not influence nicotine reinforcement behavior and likely have more subtle involvements in aversive somatic withdrawal than previously thought.
Given the high level of $\alpha2$ nAChR transcripts in the IPN, we tested whether altered neurotransmitter levels (dopamine, norepinephrine, serotonin) in the IPN could be responsible for the subtle genotype dependent differences in nicotine withdrawal. While no genotype specific effects were observed, our results demonstrated that mecamylamine-induced withdrawal assessment in nicotine treated mice disrupted the relationship between IPN tissue levels of dopamine, norepinephrine, and serotonin in predicting somatic withdrawal (paw tremors, head-shakes, backing and curls).
Discussion: Taken together, our results suggest that $\alpha2$*-containing nAChRs do not play a discernable role in nicotine reinforcement and have more subtle involvements in aversive somatic withdrawal, depending on how the assay is performed and analyzed. We speculate that other nAChRs within the IPN are involved in the disruption of neurotransmitter function in mediating nicotine withdrawal symptoms.
Disclosure: S. Lotfipour: Part 1: The first author received an investigator initiated Pfizer grant in 2010, not related to this project., Part 2: The Pfizer grant received was for greater than $10,000, Part 4: The first author received an investigator initiated Pfizer grant in 2010, not related to this project.. **C. Fowler:** None. **N. Murphy:** None. **P. Kenny:** None. **J. Boulter:** None.

90. Small and whole Transcriptome RNA Sequencing identifies Key Regulation Patterns in the Medial Prefrontal Cortex of the Alcohol Dependent Rat

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Background: MicroRNAs (miRNAs) are small interfering RNAs that regulate gene expression by binding and inhibiting target mRNAs. miRNAs expressed in the brain are critical for synaptic development and plasticity. Chronic ethanol exposure can cause

lasting changes in the medial prefrontal cortex (mPFC), in part due to changes in expression of genes involved in synaptic plasticity. Using next-generation sequencing, we cataloged whole transcriptome (WT) and miRNA expression alterations in the mPFC to further our understanding of the global regulation patterns associated with alcohol dependence.
Methods: Rats were exposed to alcohol vapor for 7 consecutive weeks. Blood alcohol concentrations (BACs) were taken weekly. Three weeks after exposure, the mPFC was harvested using the atlas of Paxinos and Watson as reference. Total RNA was isolated from the mPFC and run through the WT RNA sequencing and small RNA sequencing protocol (n = 4/group). Log2 transformation and normalization of the raw data using the Limma package from Bioconductor was used. WT and Small RNA sequence reads were mapped to rat genomic sequences (UCSC rn4) using Bowtie. Bioinformatics analysis (Ingenuity Pathway Analysis; GO analysis) and the miRNA Sanger Database were used to determine miRNA-gene interactions, pathways and functions, and networks involved in alcohol dependence.
Results: For the mPFC, we generated a total of 3.4 million and 10.3 million reads for WT and small RNA sequencing, respectively. Quantile probability plots identified that the sequencing data are normally distributed. 566 genes and 20 miRNA families were found to be differentially regulated within our data set. Gene Ontology analysis identified 150 of the 566 genes to be involved in neurological disorders. 16 genes are specifically involved in regulating the quantity of synaptic vesicles and activation of synaptic transmission. The miRNA Sanger Data base identified that the 8 top differentially expressed genes are potentially regulated by miR-200. miR-200 is known to be upregulated by oxidative stress and members of the miR-200 family regulate genes involved in neurogenesis. A subset of these mRNAs and miRNAs were further confirmed by Taqman qRT-PCR.
Discussion: Our results demonstrate a significant and lasting shift in miRNA and mRNA expression patterns in the mPFC following a history of alcohol dependence. Alcohol dependent-regulated miRNAs may contribute to long-lasting drug-induced neuroplasticity by fine-tuning regulatory pathways that modulate oxidative stress, neurotransmitter quantity and release, and synaptic plasticity. Ongoing *in vitro* and *in vivo* experiments are currently determining the functional link between our dysregulated miRNAs and their target genes.
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91. Evidence from Mouse and Man for a Role of Neuregulin 3 in Nicotine Dependence

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Background: Smoking is the largest preventable cause of death and disease in the United States, with about 46 million U.S. adults currently smoking [1]. Though there are effective medications approved by the FDA to treat nicotine addiction, roughly 80% of smokers treated with these approaches relapse within one year [2]. Interestingly, failed smoking cessation has been shown to have genetic contributions [3-5]. One protein associated with mechanisms of both gene regulation and nicotine response is the transcription factor CREB. To further investigate mechanisms underlying nicotine dependence, the current study sought to identify downstream targets of CREB and their regulation following treatment with nicotine as well as during 24 h withdrawal.
Methods: Using functional genomic approaches (chromatin immunoprecipitation (ChIP) and whole genome sequencing), CREB targets were identified following chronic nicotine administration and withdrawal. GLITR (GLobal Identifier of Target Regions) analysis was performed to assess potential target regions. Two independent biological replicates were used to validate genomic targets using ChIP, quantitative PCR and Western blotting. Validated genomic targets were then assessed for single nucleotide polymorphisms (SNPs) in the clinical population.
Results: Chronic nicotine and withdrawal differentially modulates CREB binding to the gene for Neuregulin 3 (NRG3). Quantitative PCR and Western blot analysis of saline, nicotine, and nicotine withdrawal groups in two biological replicates corroborate this finding, with NRG3 increases in both mRNA and protein following nicotine treatment and withdrawal ($p = 0.008$). Single nucleotide polymorphisms (SNPs) across *NRG3* were examined for association with prospective smoking cessation among 595 smokers smokers of European ancestry treated with transdermal nicotine in two independent cohorts. Individual SNP and haplotype analysis support association of *NRG3* SNPs and smoking cessation success; however, the function of these specific SNP markers in *NRG3* is unknown.
Discussion: These data suggest a role for NRG3 in nicotine dependence and withdrawal. NRG3 is a neural-enriched member of the EGF family, and a specific ligand for the receptor tyrosine kinase ErbB4 [6]. Of interest, genetic variation in *NRG3* has recently been implicated in risk susceptibility for schizophrenia [6-8]. Future studies in genetically modified mice for the *NRG3* gene and its cognate receptor, ERBB4, will investigate behavioral and molecular changes associated with nicotine treatment and withdrawal.
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92. *Csnk1e* is a Genetic Regulator of Sensitivity to Psychostimulants and Opioids

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Background: *Csnk1e*, the gene encoding casein kinase 1-epsilon, has been implicated in sensitivity to amphetamines. We previously

identified a quantitative trait locus (QTL) in mice for methamphetamine (MA)-induced locomotor activity near *Csnk1e*. Additionally, *CSNK1E* genetic variants have been associated with amphetamine euphoria and heroin addiction in humans. The casein kinase 1 (CK-1) family phosphorylates dopamine- and cyclic adenosine monophosphate-regulated neuronal phosphoprotein-32 (DARPP-32) and co-administration of the casein kinase 1-delta (Csnk1d)-preferring inhibitor PF-670462 with amphetamines inhibits DARPP-32 phosphorylation and the locomotor stimulant response. This suggests that CK-1 acts via the DARPP-32 pathway to influence psychostimulant sensitivity. The objective of this study was two-fold. First, we wished to narrow the QTL near *Csnk1e* that influences MA sensitivity and test for its relevance in opioid sensitivity. Second, we wished to directly test the hypothesis that *Csnk1e* regulates sensitivity to psychostimulants and opioids using knockout mice and selective pharmacological inhibition.
Methods: We conducted a genome-wide QTL mapping study of MA-induced locomotor activity (2 mg/kg, i.p.) in C57BL/6J (B6) × DBA/2J (D2)-F_2 mice and a highly recombinant F_8 advanced intercross line. We also generated and phenotyped B6.D2^{Csnk1e} and D2.B6^{Csnk1e} reciprocal congenic lines capturing *Csnk1e* (78-86.8 Mb and 78.7-81.6 Mb, respectively; *Csnk1e* = 79.25). B6.D2^{Csnk1e} were also tested for sensitivity to the mu opioid receptor agonist fentanyl (0.2 mg/kg, i.p.). Additionally, mice harboring a null allele of *Csnk1e* were tested for MA-induced locomotor activity. Last, we tested the effect of the Csnk1e-selective inhibitor PF-4800567 (40 mg/kg, i.p.) on the locomotor stimulant response to methamphetamine, fentanyl, or saline.
Results: We identified a QTL on chromosome 15 for MA sensitivity that contained *Csnk1e* (63-86 Mb; *Csnk1e* = 79.25 Mb) and further narrowed the locus to 3 Mb using reciprocal congenic mice (78.7-81.6 Mb). This locus also affected fentanyl sensitivity. *Csnk1e* knockout mice showed an increase in basal and MA-stimulated locomotor activity. The selective Csnk1e inhibitor PF-4800567 also produced an increase in MA- and fentanyl-induced locomotor activity but did not have any effect by itself. Interestingly, the enhancement of MA sensitivity with PF-4800567 depended on the genotype of congenic mice, providing further support that genetic variation in *Csnk1e* regulates the response to MA.
Discussion: These results show that a narrow genetic locus that contains *Csnk1e* is associated with differences in sensitivity to MA and fentanyl. Interestingly, the precise locus has also been identified for variation in ethanol consumption in mice. The convergence of a QTL on this locus for the response to psychostimulants, opioids, and ethanol implicates gene(s) acting via dopaminergic mechanisms. Gene knockout and selective pharmacological inhibition of Csnk1e define its role as a negative regulator of sensitivity to psychostimulants and opioids. This suggests that our previous findings regarding the Csnk1d-preferring inhibitor PF-670462 were mediated by Csnk1d and that the two isoforms exhibit opposing control over the response to drugs of abuse. In support of this hypothesis and similar to the results with *Csnk1e* knockout mice, overexpression of Csnk1d in the forebrain has been shown to increase basal and amphetamine-induced locomotor activity. Future studies will examine the role of DARPP-32 and cell type-specific expression of Csnk1e and Csnk1d in regulating sensitivity to drugs of abuse as well as their role in motivational behaviors such as pain and reward.
Disclosure: C. Bryant: None. **C. Parker:** None. **L. Zhou:** None. **C. Olker:** None. **R. Chandrasekaran:** Part 5: Pfizer, Inc. **T. Wager:**. Part 5: Pfizer, Inc. **V. Bolivar:** None. **M. Vitaterna:** Part 4: Co-investigator on a grant from Institute de Recherches de Servier. **F. Turek:** Part 1: Merck Inc, Takeda Pharmaceuticals, AstraZeneca, Servier, Sanofi-Aventis, Philips Respironics, Amylin/Lilly, Part 2: Ingram Barge Company (consulting), Part 3: Ingram Barge Company, Part 4: Merck Inc, Takeda Pharmaceuticals, AstraZeneca, American Waterways Operators, March of Dimes, NHLBI, NIA, DARPA. **A. Palmer:** None.

93. Exploratory Association Study of Genetic Variation in OPRM1 Gene and Interpersonal Dysfunction in a Personality Disorder Enriched Sample

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Background: Patients with borderline personality disorder (BPD) have very prominent difficulty in interpersonal attachment. Recently it has been suggested that this difficulty may relate to a dysregulation in opioid activity. However, few studies have explored the role of opioids in BPD and none have explored the relationship betweenthe symptom domain of interpersonal dysfunction and opioid genes in borderline personality disorder patients. Aim 1: We examined single nucleotide polymorphisms (SNPs) in the mu, delta and kappa opiate receptors and the three endogenous opiate precursor proteins proenkephalin, prodynorphin, and proopiomelanocortin as they relate to BPD diagnosis. Aim 2: In addition, as recent evidence has called into question the categorical nature of personality disorder diagnoses, we examined the domain of attachment difficulty and interpersonal aggression as it related to SNPs in opioid genes.
Methods: Subjects: We examined 47 healthy controls (HC) and 50 subjects with BPD of European American ancestry confirmed by Ancestry Informative Markers (AIMs). BPD diagnosis was assessed using the Structured Interview for DSM-IV PDs. The symptom dimension of attachment style was measured using the Experience in Close Relationship Scale (ECRI), which yielded a score for anxious and avoidant attachment styles. Genotyping: 66 SNPs from the six candidate genes (the mu, delta and kappa opiate receptors and the three precursor proteins proenkephalin, prodynorphin, and proopiomelanocortin) were genotyped on a custom-designed Illumina array, including 186 ancestry informative SNP markers. The selection of the tag SNPs was based on HapMap project Genotype data. 4/66 SNPs had insufficient variation in genotype to be included in analysis. Data analysis: Aim 1: We tested a total of 62 SNPs across 6 candidate genes for individual associations with BPD diagnosis using Chi-square tests. Aim 2: Multivariate analysis was performed to test for association between attachment scores (anxious and avoidant) and genotypes. Results were Bonferonni corrected for multiple comparisons.
Results: Aim 1: 4 SNPs were associated with BPD diagnosis (rs1799971 in the OPRM1 gene; rs10485703 and rs6045824 in the prodynorphin gene; rs9298551 in the proenkephalin gene), but none survived correction for multiple comparisons. Aim 2: Only one SNP, rs558025 in the OPRM1 gene, survived correction for multiple comparisons, with the non-ancestral (C) allele significantly associated with anxious attachment (Wilks = 0.710, approximate $F = 6.43$, $p < 0.005$ corrected). Of note, the rs558025 SNP was also significantly associated with aggression scores (ANOVA $F = 4.2$, $df = 2$, uncorrected $p = 0.016$), with the C allele showing a dose-related increase in aggression measured by a composite of two self-report instruments assessing trait aggression: the Buss Durkee Hostility Inventory (BDHI) and the Buss Perry Aggression Questionnaire (BPAQ).
Discussion: While we did not find an association between BPD diagnosis and opioid genotypes that survived correction for multiple comparisons in this preliminary sample, we did find preliminary evidence of an association between genetic variation in the OPRM1 gene and symptoms of interpersonal dysfunction, including interpersonal aggression and attachment anxiety, in a sample enriched with personality disordered subjects. This is interesting as genetic variability in this gene has previously been reported in a human sample of psychiatric patients and controls and in a homologous gene in infant primates. Moreover, the finding of dimensional endophenotypes such as attachment

anxiety or aggression showing a stronger association with the genotype than the categorical diagnosis of BPD supports the dimensional approach to personality disorder diagnoses.
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94. Exploratory Association Study of 130 Candidate Genes in Patients with Borderline Personality Disorder and Healthy Controls

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Background: Growing evidence demonstrates a genetic vulnerability for borderline personality disorder (BPD), with twin studies showing substantial heritability scores of up to 0.70. However, no genome-wide association study has been conducted to identify potential loci associated with BPD diagnosis, and only one family-based linkage study is available to date. Most studies have focused on only one or two candidate genes at a time, mainly in the serotonergic and dopaminergic systems. We aimed to identify single nucleotide polymorphisms (SNPs) associated with BPD diagnosis among a set of 1533 SNPs from 130 candidate genes selected for their relevance in addiction, mood disorders and impulsive behaviors, including genes from signaling networks, stress/endocrine pathways, and key neurotransmitter systems, including dopamine, serotonin, glutamate, GABA and acetylcholine.
Methods: Subjects: The full data set consisted of 156 healthy controls (HC) and 181 subjects with BPD, including a European American subsample (HC n = 89; BPD n = 86) confirmed by Ancestry Informative Markers (AIMs). BPD diagnosis was assessed using the Structured Interview for DSM-IV PDs. Genotyping: 1533 SNPs from 130 candidate genes were genotyped on a custom-designed Illumina array, including 186 ancestry informative SNP markers. The selection of the tag SNPs was based on HapMap project Genotype data. The goal was to obtain a panel of markers able to extract full haplotype information for the selected candidate genes. Genotyping was carried out following Illumina GoldenGate assay protocols and the arrays were imaged on an Illumina Beadstation GX500. Data preprocessing: The following quality control steps were performed on these genotype data using the PLINK software package. Subjects were excluded from the analysis if they had missing data rates $\geqslant$20%; SNPs were excluded if they had Minor Allele Frequency (MAF) $<$ 0.05, missing data rates $\geqslant$5%, or Hardy-Weinberg equilibrium P-values $\leqslant 5e^{-5}$. After the quality control procedure, 171 subjects of homogeneous ancestry (HC n = 88; BPD n = 83) and 914 markers remained in the analysis. Data analysis: Logistic regression analysis was performed to test for association with BPD diagnosis via four genetic models: dominant, recessive, additive and genotypic. The regression was adjusted for sex and the 6 AIMs eigenvalues from the Principal Component Analysis. Corrections for multiple comparisons were done by using 1000 permutations.
Results: Only one SNP, rs1611131 in the dopamine beta hydroxylase gene, survived correction for multiple comparisons, with the C allele significantly associated with BPD diagnosis (Dominant model: OR = 3.928; Chi Square = 3.921; uncorrected p = 0.00009; corrected p = 0.03497; Additive model: OR = 2.859; Chi Square = 3.773; uncorrected p = 0.00016; corrected p = 0.05095;). The rs1611131 SNP was found to be in linkage disequilibrium (LD) with the rs2073833 SNP of the dopamine beta hydroxylase gene. In a subsequent haplotype analysis, the rs2073833_rs1611131 CC vs CA and AA haplotype was found to be significantly associated with BPD diagnosis (OR – 95%CI: 2.6 (1.5 – 4.4); Likelihood ratio test: chi-square = 12.6; df = 1; p = 0.0004).
Discussion: Although dopaminergic drugs (i.e., antipsychotics) are often used for the treatment of BPD, and there is some evidence of a dysregulation in dopaminergic activity among BPD patients, few studies have explored the role of dopaminergic genes in BPD. We found a significant association of the C allele of the rs1611131 SNP in the dopamine beta hydroxylase gene with BPD diagnosis. Replication of this finding is warranted. Future studies are needed to clarify the role of this SNP and investigate the surrounding loci for functional effects on dopaminergic transmission.
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95. Gene $\times$ Disease Interactions on Stress Reactivity in Cocaine Addiction

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Background: Allele variations in themonoamine oxidase A (MAOA) and serotonin transporter (SERT, or SLC6A4) genotypes have been associated with increased vulnerability to stressful environments; as such, these polymorphisms may play an interactive role in the course of addiction, a disease that is associated with increased exposure to stress. We therefore examined genetic variations as a function of the trait Stress Reaction in individuals with cocaine use disorders (CUD) and healthy controls. We hypothesized that the low-MAOA and short SERT alleles will have traits of increased Stress Reaction within CUD.
Methods: Subjects were 19 individuals with CUD and 23 healthy controls, all Caucasian or Hispanic, who completed the Multidimensional Personality Questionnaire. High scorers on the Stress Reaction subscale describe themselves as irritable, overly sensitive to minor frustration and emotionally liable. The individuals were genotyped for MAOA with "high" or "low" alleles and SERT SLC6A4 for the presence of a short (vs. long/long) allele. The impact of diagnosis and the two polymorphisms on Stress Reaction was tested by 1) comparing CUD with controls, and 2) testing diagnosis-by-MAOA/SERT interactions. General Linear Models were conducted controlling for age and with a threshold of $p < .05$, one-tailed.
Results: There was a significant main effect of addiction such that CUD reported more Stress Reaction than controls ($F_{1,38} = 8.3$, $p = 0.007$). In addition the following interactions were significant: **1)** SERT genotype $\times$ diagnosis interaction, where the presence of the short allele was associated with higher Stress Reaction within CUD ($F_{1,28} = 8.5$, $p = 0.007$); and **2)** MAOA genotype $\times$ diagnosis interaction where the low allele was associated with higher Stress Reaction within CUD ($F_{1,38} = 4.7$, $p = 0.036$). The three-way interaction (MAOA $\times$ SERT $\times$ diagnosis) showed a trend for the combination of the short SERT and low MAOA alleles to confer increased Stress Reaction in CUD ($F_{7,28} = 1.8$, $p = 0.059$).
Discussion: The results show that, as a group, individuals with cocaine addiction report more stress reactivity than healthy controls. However, genetic variation that is known to affect reactivity to stressors is operating within cocaine addiction to distinguish a subgroup that reports high trait reactivity to stress. It was already demonstrated that CUD with the low MAOA genotype have less gray matter in the Orbitofrontal cortex as compared with

CUD with high MAOA genotype. Thus, enduring patterns of Stress Reaction in carriers of low MAOA and short SERT alleles may modulate the course of illness rendering these individuals vulnerable to increased drug use and to relapse. Since stress reactivity is implicated in relapse a better understanding of the genetic factors that enhance stress reactivity could be used to tailor relapse prevention interventions in CUD.
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96. TTC12-ANKK1-DRD2 and CHRNA5-CHRNA3-CHRNB4 influence Different Pathways leading to Smoking Behavior from Adolescence to Mid-Adulthood

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Background: CHRNA5-CHRNA3-CHRNB4 and TTC12-ANKK1-DRD2 gene-clusters influence smoking behavior. Our aim was to test developmental changes in their effects as well as the interplays between them and with nongenetic factors.
Methods: Participants included 4762 subjects from a general population-based, prospective Northern Finland 1966 Birth Cohort (NFBC 1966). Smoking behavior was collected at age 14 and 31 years. Information on maternal smoking, socioeconomic status, and novelty seeking were also collected. Structural equation modeling was used to construct an integrative etiologic model including genetic and nongenetic factors.
Results: Several single nucleotide polymorphisms in both gene-clusters were significantly associated with smoking. The most significant were in CHRNA3 (rs1051730, $p = 1.1 \times 10(-5)$) and in TTC12 (rs10502172, $p = 9.1 \times 10(-6)$). CHRNA3-rs1051730[A] was more common among heavy/regular smokers than nonsmokers with similar effect-sizes at age 14 years (odds ratio [95% CI]: 1.27 [1.06-1.52]) and 31 years (1.28 [1.13-1.44]). TTC12-rs10502172[G] was more common among smokers than nonsmokers with stronger association at 14 years (1.33 [1.11-1.60]) than 31 years (1.14 [1.02-1.28]). In adolescence, carriers of three-four risk alleles at either CHRNA3-rs1051730 or TTC12-rs10502172 had almost threefold odds of smoking regularly than subjects with no risk alleles. TTC12-rs10502172 effect on smoking in adulthood was mediated by its effect on smoking in adolescence and via novelty seeking. Effect of CHRNA3-rs1051730 on smoking in adulthood was direct.
Discussion: TTC12-ANKK1-DRD2s seemed to influence smoking behavior mainly in adolescence, and its effect is partially mediated by personality characteristics promoting drug-seeking behavior. In contrast, CHRNA5-CHRNA3-CHRNB4 is involved in the transition toward heavy smoking in mid-adulthood and in smoking persistence. Factors related to familial and social disadvantages were strong independent predictors of smoking
Disclosure: F. Ducci: none. **M. Kaakinen:** None. **J. Veijolai:** None. **M. Isohannii:** None. **G. Schumann:** None. **M. Jarvelin:** None.

97. Linkage Analyses of Stimulant dependence, craving and heavy use in American Indians

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Background: Stimulants [STIM] (methamphetamine [MA] and cocaine [COC]) are the most commonly used illicit drugs worldwide second to cannabis use. Recent surveys indicate that MA is the fastest-growing illicit drug of choice, particularly in the Western United States and Canada, leading some to describe the MA problem as an "epidemic"..Studies that have evaluated the role of genetic and environmental risk factors on stimulant abuse or stimulant dependence in twin samples have found heritability estimates that range from 0.39 to 0.79. Despite these substantial heritability estimates, identifying genetic loci that confer risk for stimulant misuse disorders has been difficult given that the genetic architecture underlying these disorders and substance use disorders in general appears to be polygenic. This study's aims were to map loci linked to stimulant dependence, heavy use, and craving in an American Indian community at high risk for substance dependence.
Methods: DSM diagnosis of stimulant dependence, as well as indices of stimulant "craving" and "heavy use", were obtained using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). Genotypes were determined for a panel of 791 micro-satellite polymorphisms in 381 members of multiplex families using SOLAR.
Results: Stimulant dependence, stimulant "craving" and "heavy stimulant use", were all found to be heritable. Analyses of multipoint variance component LOD scores, failed to yield evidence of linkage for stimulant dependence. For the stimulant "craving" phenotype, linkage analysis revealed a locus that had a LOD score of 3.02 on chromosome 15q25.3-26.1 near the nicotinic receptor gene cluster. A LOD scores of 2.05 was found at this same site for "heavy stimulant use". Additional loci with LOD scores above 2.00 were found for stimulant "craving" on chromosomes 12p13.33-13.32 and 18q22.3.
Discussion: These results corroborate the importance of "craving" as an important phenotype that is associated with regions on chromosome 12, 15 and 18,that have been highlighted in prior segregation studies in this and other populations for substance dependence-related phenotypes. In conclusion, these data represent the first linkage analysis of amphetamine-related phenotypes in any population. The results suggest that several areas of the genome may harbor genes that modulate level of addiction to stimulants. (supported by DA 019333, AA10201).
Disclosure: C. Ehlers: None. I. Gizer: None. D. Gilder: None. K. Wilhelmsen: None.

98. HPA Axis Response to Acute Stress and Hippocampal Expression of Stress-Related Genes in Alcoholics and Cocaine Addicts

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Background: Alcohol and drug addiction have been associated with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis stress system and such changes are associated with addiction relapse outcomes. The purpose of this study, conducted in alcoholics and cocaine addicts, was firstly to investigate genetic influences on HPA axis response to acute experimental stress and secondly to measure expression of the same genes in postmortem hippocampus, a brain region implicated in both stress and addiction.
Methods: The first study sample included one-month abstinent, inpatient alcoholics (AD) ($N = 51$, 8 women) and cocaine addicts (CO) ($N = 48$, 20 women) together with 57 healthy controls (29 women). All subjects identified a personal highly stressful event and a personal neutral, relaxing event which were used to develop audiotaped scripts. Blood cortisol and ACTH levels were measured at baseline and at various time points following exposure to script-driven imagery of the stress and neutral events conducted in the early morning to capture the naturally elevated cortisol followed

by the diurnal decline. Haplotype tagging SNPs were genotyped using the Illumina GoldenGate platform across six genes: the CRH gene complex (CRH, CRHBP, CRHR1), NR3C1 and FKBP5 respectively encoding the glucocorticoid receptor and its key co-chaperone, and NPY, encoding the anxiolytic neuropeptide Y. Outcome measures were area-under-the-curve (AUC) cortisol and ACTH in the stress and neutral conditions. In the second, all-male study sample of 8 alcoholics, 8 cocaine addicts and 8 controls, RNA-Seq was used to quantify mRNA transcripts from the same six genes.

Results: There were sex and group differences in cortisol and ACTH levels. The female controls showed a greater decline over time in cortisol levels compared with male controls. The female controls and CO showed no cortisol response to the stress condition compared with the neutral condition. In contrast, male controls and CO showed a cortisol increase in response to stress. The male AD had lower baseline cortisol compared with CO and controls and no cortisol response to stress. Male CO and AD had higher ACTH levels than male controls and women of all groups. Four SNPs were in strong linkage disequilibrium across the CRH gene. The rs6472257 minor allele was associated with increased AUC cortisol in the stress, but not the neutral condition across both sexes and all groups ($p = 0.007$). Despite the smaller sample size, the effect for rs6472257 was more marked in the group of men ($p = 0.005$) and there were also significant effects in the same direction of two other linked SNPs, rs6996265 and rs3176921 ($p < 0.05$). The CRH SNPs rs6996265, rs3176921 and rs5030875 were associated with increased ACTH AUC in both the stress ($p = 0.006$, $p = 0.004$, $p = 0.03$) and neutral conditions $p = 0.003$, $p = 0.034$, $p = 0.047$) across all 3 groups and both sexes. The minor homozygote of NPY SNP rs16148 was associated with lower AUC cortisol levels in response to stressful, but not neutral, imagery in male AD and CO and male and female controls ($p = 0.036$) with a trend effect on AUC ACTH under stress imagery ($p = 0.071$). There were no significant results for CRHBP, CRHR1, NR3C1 or FKBP5 SNPs. In the postmortem hippocampal samples, CO showed lower expression of CRH ($p = 0.006$), CRHBP ($p = 0.009$) and NPY ($p = 0.024$) and higher expression of FKBP5 ($p = 0.032$) but there were no gene expression changes in alcoholics.

Discussion: We have shown that CRH and NPY genetic variation has an effect on cortisol and ACTH levels that is independent of group and sex. We have identified differences between CO and AD in both HPA axis response to acute stress and in hippocampal expression of stress genes.

Disclosure: M. Enoch: None. **Z. Zhou:** None. **D. Mash:** None. **Q. Yuan:** None. **D. Goldman:** None. **R. Sinha:** None.

99. Galanin Receptor 1 (*GALR1*) SNP is Associated with Craving and Smoking Relapse

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Background: Galanin modulates dopaminergic neurotransmission in the mesolimbic dopamine system, thereby influencing the rewarding effects of nicotine. Variants in the *GALR1* gene have been associated with nicotine dependence and retrospective craving severity (Jackson *et al.*, *Neuropsychopharmacology*, 2011; Lori *et al.*, *Neuropsychopharmacology*, 2011). This study investigated the association of rs2717162, based on the *Lori et al.* study, with quitting success and post-quit craving symptoms among treatment-seeking smokers.

Methods: rs2717162 was genotyped in 410 smokers of European ancestry enrolled in a double-blind randomized placebo-controlled clinical trial of bupropion for 10 weeks. The primary endpoint was abstinence (7-day point prevalence, biochemically confirmed). Cravings and urges to smoke were assessed on the target quit day (TQD). Generalized estimating equation (GEE) regression was used to assess genotype associations with abstinence at the end of treatment (EOT) and 6-month follow-up, controlling for treatment, nicotine dependence, and sex.

Results: There was a significant decrease in the odds of quitting success with the presence of at least one minor (C) allele (OR = 0.52; 95% CI = 0.33, 0.83; $p = 0.005$). Although the main effect of treatment was significant, genotype by treatment and genotype by time point (EOT vs. 6M) interactions were not significant. Cox regression analysis revealed a positive association of the C allele with time to first cigarette ($p = 0.017$). Linear regression on TQD craving, controlling for the effect of treatment, revealed a positive association of the rs2717162 C allele with craving ($p = 0.002$). Both genotype and craving were associated independently with relapse.

Discussion: Variation in *GALR1* SNP rs2717162 is associated with quitting success, time to first cigarette, and post-quit craving in treatment seeking smokers, independent of bupropion vs. placebo treatment. Consistent with preclinical studies and two prior retrospective association studies, these prospective cessation data provide support for further research on *GALR1,* nicotine dependence, and quitting success.

Disclosure: A. Gold: None. **E. Wileyto:** Part 1: Dr. Wileyto has consulted for Pfizer. **C. Jepson:** None. **A. Lori:** None. **J. Cubells:** Part 1: Dr. Cubells has served as a consultant for Dykema, Gossett, LLP and Barnes and Thornburg, LLP., Part 4: Dr. Cubells has received research funding from Roche and Seaside Therapeutics. **C. Lerman:** Part 1: Dr. Lerman has served as a consultant for Pfizer, GlaxoSmithKline, AstraZeneca, and Targacept, Part 4: Dr. Lerman has received research funding from AstraZeneca, GlaxoSmithKline, and Pfizer.

100. Association Of DRD4 Exon 3 VNTR And Alcohol's Effects On Temporal Cognition

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Background: Time estimation is influenced by many factors and can affect behavior. For example when hungry and waiting for a table in a restaurant and time appears to be passing more slowly than reality, one might be more likely to leave and find another restaurant that has seating immediately available. There is a mixed literature on the effects of alcohol on time estimation; some studies find that alcohol results in overestimates in time and others find underestimates. In acute tobacco administration studies a relationship between urge to smoke and time estimation has been reported. Variation in the Dopamine D4 receptor gene (*DRD4*) has been associated with differential urges for multiple substances of abuse (including alcohol and tobacco). We hypothesized that alcohol administration would influence time estimates and that variation in the DRD4 gene would moderate these effects.

Methods: 62 non-alcoholic participants underwent 2 alcohol administration sessions to reach targeted BACs of 0.04 and 0.08 respectively. Participants were asked to estimate when a 47 second interval had passed. Participants estimated this interval 5 times during each alcohol administration: at baseline (BAC = 0), on the ascending limb, at or near peak BAC, on the descending limb, and after returning to BAC of 0. Participants were genotyped for the *DRD4* exon 3 VNTR and grouped as short homozygotes (both alleles were <7 repeats) and long carriers (at least one allele was > 6 repeats).

Results: For the first session (target BAC of 0.04), the baseline (pre-alcohol) actual time intervals were significantly less than the next three estimates (post-alcohol). For the second session (target

BAC of 0.08), the baseline time estimation was significantly less than the next two estimates. Final time estimates were less than peak estimates during both sessions. Baseline and final time estimates were equivalent; an inverse U function was apparent. DRD4 short homozygotes indicated longer time intervals in the time estimation task compared to DRD4 long homozygotes: across the first session (Day 1, target BAC of .04) $t(60) = 2.43$, $p = .02$ [53.0 (17.8) sec vs. 41.3 (15.1) sec] and across the second session (Day 2, target BAC of .80) $t(43) = 2.30$, $p = .03$. [54.2 (15.9) sec vs. 42.8 (12.6) sec]. Significant differences based on DRD4 status were also exhibited for 0.04 session at baseline. Statistical trends were apparent based on DRD4 status for Day 1 ascending limb, Day 1 descending limb, Day 2 baseline, and Day 2 ascending limb.
Discussion: Although in general alcohol administration resulted in an over indication of the time interval, variation in the *DRD4* gene was associated with differences in time estimation at both alcohol doses. Consistent with hypotheses, presence of the long allele was associated with an underestimation of time (i.e., time appeared to pass more slowly than reality; the internal clock was moving more slowly than the actual clock). Such an underestimation of time may impact the likelihood of consuming additional alcoholic beverages in an ad lib condition as the drinker may be 'impatient' for the rewarding effects of additional alcohol consumption. Similarly, since time estimation is critical to the estimation of distance traveled while driving, changes in the speed of one's internal clock may have safety implications for intoxicated drivers' ability to drive safely even at breath alcohol levels that are below the legal limit or 0.08.
Disclosure: J. McGeary: None. **R. Swift:** Part 1: Alkermes, Part 4: Eli Lilly. **W. Zywiak:** None. **A. Grenga:** None. **J. Kim:** None. **J. Murphy:** None.

101. Dopaminergic and Cholinergic Genetic Variation moderates Smoking-Induced Striatal Dopamine Release

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Background: Genetic variation in smoking-induced dopamine (DA) release may be relevant to clinical smoking phenotypes, including success with smoking cessation. We previously reported genetic variants in dopaminergic systems that predict inter-individual variation in smoking-induced DA release as measured by positron emission tomography (PET). In our prior analysis, gene variants in the dopamine transporter (DAT), dopamine DRD4 receptor and the catabolic enzyme COMT were associated with greater smoking-induced DA release in the striatum of tobacco-dependent smokers. Nevertheless, variation in DA dynamics in smokers remains only partially explained. We hypothesized that more direct moderators of DA signaling in the striatum would include genetic polymorphisms in the downstream target of synaptic DA, the dopamine D2 receptor (DRD2), and the proximal site of nicotine action in the brain, nicotinic acetylcholine receptors (nAChRs).
Methods: 102 otherwise healthy adult (21-65 years old) tobacco-dependent smokers (15-40 cigarettes per day) were interviewed with standardized questions; completed rating scales related to cigarette usage, mood, and personality; had blood drawn for genotyping; underwent pre- and post-cigarette smoking [^{11}C]raclopride PET scans; and underwent structural magnetic resonance imaging (MRI) to aid in interpretation of the PET scans. PET scanning and administration of rating scales were completed on a single afternoon; subjects underwent structural MRI on a separate morning within one week of PET scanning. Exclusion criteria included a history of any Axis I psychiatric or substance abuse/dependence diagnosis other than nicotine dependence on the Structured Clinical Interview (SCID) for *DSM-IV*. We measured the association of candidate gene markers with smoking-induced change in [^{11}C]raclopride binding potential as a proxy for DA release in the ventral striatum. Previously, only four functional polymorphisms in gene candidates were tested. In the current study, we genotyped tag single nucleotide polymorphisms (tSNPs) to represent the complete common variation across DRD2 and six acetylcholine receptor subunit genes (CHRNA3, CHRNA4, CHRNA5, CHRNA7, CHRNB2, and CHRNB4). tSNPs were selected to capture all alleles occurring at a minor allele frequency of 10% or greater and at an r^2 of 0.8 or greater using the Broad Institute Tagger software. Genotypes were generated using the Life Sciences Taqman genotyping platform with the substitution of Qiagen Type-it Fast SNP Probe PCR Kit in accordance with manufacturer's specified protocols. Overall univariate analysis of variance (ANOVA) was performed; marker alleles were tested as between-subject factors without interactions for association with percent change in binding potential in the volume-weighted mean of left and right ventral caudate (VCD) regions of interest (including nucleus accumbens). We further correlated genetic variants and imaging data with behavioral measures of craving.
Results: Homozygotes for the common allele (GG) at an intron 2 polymorphism in the alpha 7 cholinergic receptor (CHRNA7, rs12915695) showed greater than 3X reduction in radiotracer binding potential, indicating significantly greater smoking-induced DA release, compared to carriers of the minor A-allele (-10.8% vs. -3.0% respectively, $p = 0.002$). This result remained significant after correction for multiple comparisons. Similarly, CC homozygotes at a variant in the promoter of the alpha 4 cholinergic receptor (CHRNA4, rs755203) had almost twice the reduction in radiotracer binding (-10.7% vs. -5.7%) as carriers of the minor T-allele ($p = 0.047$). This SNP is in modest linkage disequilibrium (LD) with a published marker associated with nicotine dependence.
Discussion: These data suggest that structural or functional variation in midbrain nAChRs contributes to differential smoking-induced striatal DA release. Findings at nicotinic alpha 4 and alpha 7 cholinergic receptor subunits are consistent with animal and *in vitro* studies implicating these subunits in DA-mediated reinforcement associated with smoking. Together with our earlier data showing moderation of DA release by DAT, DRD4, and COMT gene variants, we again show that common gene variants, this time in nicotinic cholinergic receptor subunits, appear to exert significant effects on smoking-related DA release. A comprehensive understanding of genetic moderators of nicotine reward and risk for dependence may facilitate the development and individualization of successful treatment strategies.
Disclosure: E. Nurmi: None. **K. Mallya:** None. **T. Levin:** None. **K. Ta:** None. **J. La Charite:** None. **J. McCracken:** Part 1: Roche, Novartis, BioMarin, PharmaNet, Part 4: Seaside Therapeutics, Bristol Myers Squibb. **A. Brody:** None.

102. Genome-Wide Association Study of *d*-Amphetamine Response in Healthy Human Participants identifies Association with Cadherin 13 (*CDH13*)

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Background: Both the subjective response to amphetamine and risk for amphetamine addiction are known to be heritable traits. Furthermore, epidemiological data show that the subjective response to drugs is a risk factor for drug addiction; however, it is not known whether this correlation is due to genetic or

environmental factors. Althought there have been several genome-wide association studies (GWAS) of drug addiction there have been no GWAS of the acute subjective responses to stimulant drugs.
Methods: We performed a GWAS of response to *d*-amphetamine in 381 human participants. Acute amphetamine response was measured using a double-blind, placebo-controlled, within-subjects design. We used sparse factor analysis to reduce the dimensionality of the response data to 11 highly-interpretable factors.
Results: We identified a strong association between a subjective response factor and a SNP (rs3784943) in the cadherin 13 gene (*CDH13*; $P = 4.68 \times 10^{-8}$), which has previously been associated with methamphetamine dependence.
Discussion: Our results provide the first unbiased evidence that genes influencing the subjective responses to amphetamine overlap with genes that influence the risk for addiction. These findings suggest an underlying molecular mechanism of drug response and addiction. More broadly, our results suggest that intermediate pharmacogenomic phenotypes such as acute subjective response may be more tractable targets for GWAS as compared to clinical diagnoses such as drug addiction.
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103. Quit Success Genotype Score predicts Success and Lower/Slower Paced Involvement with Common Addictive Substances

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Background: Complex genetics of common illnesses and treatment responses are captured to varying extents by genotype scores that combine weighted data from allelic variants at many loci. We have developed such a genetic score for individual differences in abilities to quit smoking. In prior work we prospectively applied this v1.0 score to participants in a smoking cessation clinical trial in which nicotine replacement (NRT) dose was randomly assigned to individuals. In this previous trial, the score successfully predicted cessation as an interaction with NRT dose. We now report results of application of this v1.0 score to two novel samples: a) successful quitters *vs* matched nonquitters from a new smoking cessation trial in which NRT dose was matched to the intensity of smoking and b) a community based sample whose addictive substance use was monitored periodically from ages 14-24.
Methods: Gentotype scores from all participants in the smoking cessation clinical trial who achieved continuous abstinence for 11 weeks were compared to those for twice the number of matched nonquitters who failed to achieve 11 week continuous abstinence. Matching was done on the basis of gender, ethnicity/race and assigned arm of the adaptive treatment trial. DNAs were genotyped using Affymetrix 6.0 arrays, and v1.0 scores calculated based on weighted contributions from previously-defined "quit success" alleles at 12,058 SNPs. We compared quit success scores from quitters to those from nonquitters using t tests, and quit success of individuals in the upper terclie of quit success scores to those in the lower tercile using chi square tests. Participants in a longitudinal followup study of subjects from a first grade prevention intervention trial were questioned periodically about their last year substance use beginning in eighth grade. A summed score was developed for use of tobacco, marijuana and alcohol. Latent class growth mixture modeling (implemented in M+) identified three classes of participants based on their fit with three developmental trajectories of use of these common addictive substances, and provided probabilities of class membership (with race/tehnicity and gender as covariates) for each of the 555 individuals for whom we had clinical followup and DNA. v1.0 scores were calculated from Affymetrix 6.0 genotypes of these individuals, as noted above. Primary preplanned analysis tested differences in class membership probabilities for individuals in the upper vs lower terciles of the distribution of v1.0 scores. Other analyses included assessing the significance of v1.0 scores when they were added as covariates to the lateng class growth analyses.
Results: The v1.0 score predicted ability to quit in the new sample of smokers ($p = 0.00056$, area under ROC curve 0.657). About 12% of individuals in the lower tercile of v1.0 scores quit; about 43% of individuals in the upper tercile of v1.0 scores quit. The v 1.0 score also displayed significant association with the pace of change in use of common addictive substances during childhood and adolescence. Latent class growth analyses of the community-based, developmentally-assessed sample identified three latent classes based on substance use. In accord with expectations, higher v1.0 scores were associated with higher probabilities that a subject would be a member of a latent class that displayed slower and less robust development of use of common addictive substances during adolescence ($p = 0.0004$), and lower probabilities of membership in a class that reported rapidly escalating use ($p = 0.001$).
Discussion: These results support the ideas that: a) aid for smoking cessation can be personalized based on genetic predictors of outcome and b) genetic influences on remission of an active addiction may overlap with those that influence the rate at which addictive substance use is taken up during development. This work provides one of the first examples of successful prediction of ability to achieve a therapeutically-meaningful endpoint, smoking cessation, based on a complex genotype score. The area under the receiver operating characteistic curve compares well with results in other complex disorders. This work also provides some of the first evidence that genetic influences on abilities to quit use of an addictive substance overlap with those that help to determine the way in which use of addictive substances is taken up during development.
Disclosure: G. Uhl: Part 1: n/a, Part 2: NIH Potential to generate royalties: Drs Uhl and Rose are coinventors of patent submitted by Duke University for SNPs that predict smoking cessation success. **D. Walther:** none. **W. Eaton:** Part 2: Johns Hopkins Bloomberg School of Public Health. **N. Ialongo:** Part 1: N/A, Part 2: Johns Hopkins Bloomberg School of Public Health. **J. Rose:** Part 1: Consultant, Philip Morris International, Part 2: Duke University Consultant, Philip Morris International Potential to generate royalties: Drs Uhl and Rose are coinventors of patent submitted by Duke University for SNPs that predict smoking cessation success. Dr Rose is the inventor of a nicotine delivery system recently licensed to Philip Morris international, Part 3: (see above), Part 4: See above.

104. Effects of *PPP1R1B* Genetic Variation on In Vivo Human Central Dopamine System Function

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Background: Central dopamine dysfunction is a key feature and broad therapeutic target of several debilitating, heritable neuropsychiatric illnesses, including schizophrenia, which is associated with abnormal striatal presynaptic dopamine synthesis and complex genetic risk architecture. The salient molecular determinants

of central dopamine systems function in humans remain largely unclear, hindering progress toward more specific treatments. DARPP-32 is a critical regulator of postsynaptic dopamine signaling cascades that has been implicated in schizophrenia. A frequent 7-marker haplotype in the gene coding for DARPP-32, *PPP1R1B*, predicts greater mRNA expression in postmortem prefrontal cortical samples, better cognitive performance, and, by MRI, reduced striatal volumes and activation, and increased structural and functional prefrontal-striatal connectivity as well as risk for schizophrenia. The genetically driven disparity in *PPP1R1B* expression may give rise to the cognitive and neuroimaging associations via differences in dopaminergic system functioning. However, despite murine experiments showing altered striatal dopamine release resulting from specific DARPP-32 gene mutations, there is no in vivo evidence directly yoking common variation in *PPP1R1B* to dopamine function in the human brain.
Methods: To fill this key knowledge gap, we studied 82 healthy Caucasian adults (mean age 33 ± 9, range 18 to 49, 37 female) who underwent both genotyping by Taq-Man 5′-exonuclease assay for the aforementioned haplotype and ^{18}F-FDOPA PET, a robust assay of dopamine synthesis and storage. Carbidopa was administered prior to imaging to minimize peripheral tracer degradation. Following injection of 16 mCi of ^{18}F-DOPA, emission images were acquired dynamically over 90 minutes, attenuation-corrected, realigned, coregistered to each subject's structural MRI, warped to standard space using the DARTEL algorithm, and smoothed with SPM8 software. The kinetic rate constant Ki for tracer uptake was calculated using the Patlak method with an occipital reference region. Whole-brain voxel-wise regression analysis of Ki maps used the number of risk-associated haplotype copies each individual possessed (0, 1, or 2) as the regressor of interest and age and sex as nuisance covariates. Haplotypes were generated using PHASE software.
Results: There were 52 homozygotes, 27 heterozygotes, and 3 subjects with no copy of the *PPP1R1B* haplotype of interest. The *PPP1R1B* haplotype was associated with lower Ki in roughly symmetrical bilateral clusters located in medial dorsal basal ganglia, including globus pallidus with extension into the caudate head (uncorrected voxel-level $p < 10^{-4}$).
Discussion: We demonstrate for the first time that common variation in *PPP1R1B* predicts basal ganglia dopamine function in the living human brain. Insofar as DARPP-32 predominantly influences signaling cascades in cells receiving dopaminergic input, these data provide support for a presynaptic adaptation to genetically-determined postsynaptic dopamine system characteristics, in line with previous animal models. Further studies in patients are merited to better delineate potential intersections between these findings and dopaminergic pathology in schizophrenia.
Disclosure: D. Eisenberg: None. **J. Masdeu:** None. **P. Kohn:** None. **A. Ianni:** None. **E. Baller:** None. **B. Kolachana:** None. **D. Weinberger:** None. **K. Berman:** None.

105. Acamprosate Acts as a Partial Agonist of the NMDA Receptor: Evidence from a Spectroscopy Study in Schizophrenia

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Background: Multiple lines of evidence suggest that the glutamatergic system is involved in schizophrenia pathology. Proton magnetic resonance spectroscopy (^{1}H-MRS) studies have offered support for this hypothesis. Levels of glutamate and/or glutamine are higher in the anterior cingulate region (ACC) of people with schizophrenia, who are either early in their illness or experiencing an acute symptom exacerbation, compared to healthy controls. However, these levels are lower than controls when schizophrenia becomes chronic and symptoms are stable. Furthermore, our group has shown that ACC glutamate and glutamine (Glu + Gln) levels also vary based on history of alcohol use. These observations indicate that a partial agonist of glutamate receptors may be clinically useful in the treatment of people with schizophrenia, both for symptoms of the illness and for co-occurring alcohol use disorders. The drug acamprosate behaves as a partial agonist of the glutamatergic N-methyl-D-aspartate (NMDA) receptor in vitro. This ^{1}H-MRS study investigated whether acamprosate would behave as a partial agonist of the NMDA receptor in vivo in people with schizophrenia. It was hypothesized that change in Glu + Gln levels would vary depending on baseline Glu + Gln levels; higher baseline levels predicting a decrease, and lower baseline levels predicting an increase, in Glu + Gln.
Methods: Participants were 24 clinically stable outpatients with DSM-IV schizophrenia/ schizoaffective disorder. ^{1}H-MRS used a Philips ACS-NT 3T scanner (Best, Netherlands) with an 8-channel head coil. Spectra were obtained at baseline and after a 2 week challenge of open-label acamprosate, 1998mg/d. Spectra were acquired from a 2x2x2cc voxel encompassing the bilateral ACC using point-resolved pulse sequence (PRESS; TR = 2000ms, TE = 35ms, 1024 points, 2000Hz spectral width, 256 averages, scan time 8min 34sec). Water suppression was achieved with variable pulse powers and optimized relaxation delays (VAPOR) presaturation pulses. Spectra were analyzed using the fully automated, standard curve-fitting software, LCModel. Metabolite concentrations were normalized to unsuppressed water peaks. Linear regression was used to examine the relationship between change in Glu + Gln and baseline Glu + Gln levels.
Results: Mean baseline Glu + Gln levels were 8.86 ± 1.04 (range: 7.11 to 11.11). Baseline Glu + Gln levels in the ACC region significantly predicted Glu + Gln change in response to the acamprosate challenge ($\beta = -0.42$, $t(23) = -2.157$, $p = 0.04$). The negative slope (β) indicates individuals with low baseline Glu + Gln in the ACC had an increase in the level in response to acamprosate, whereas those with high baseline Glu + Gln had a decrease.
Discussion: To our knowledge, this is the first in vivo evidence that acamprosate acts as a partial agonist within the glutamatergic system. As such, acamprosate may be a useful agent in stabilizing the glutamatergic system in schizophrenia regardless of phase-of-illness and may offer unique benefit to individuals with schizophrenia and co-occurring alcohol use disorders.
Disclosure: B. Fischer: None. **L. Rowland:** None. **W. Keller:** None. **H. Holcomb:** None. **R. Buchanan:** Part 1: Abbott, Astra-Zeneca, Cypress Bioscience, Merck, Pfizer, Roche, Solvay, Wyeth, Sanofi-Aventis, Glaxo-Smith-Kline, Otsuka, Cephalon, Part 4: Ortho-McNeil/Janssen Scientific Affairs.

106. Expression Profile of Metabotropic Glutamate Receptors in the Human Post Mortem Schizophrenia Brain

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Background: Schizophrenia is a brain disease of obscure etiology. One of the hypotheses posits a reduction of glutamatergic neurotransmission in this illness, based on the clinical observation that N-methyl-D-aspartate (NMDA) receptor antagonists produce a syndrome similar to schizophrenia. NMDA receptor function can be modulated by the metabotropic glutamate receptors (mGluRs, GRMs), a family of proteins divided into 3 groups that differ in distribution, function and physiologic role. The mGluRs are divided in to three groups - group I (mGluR1 and 5), group II

(mGluR2 and 3) and group III (mGluR4, 6, 7, and 8). There is substantial evidence suggesting a role for mGluRs, particularly groups I and II, in the pathophysiology of schizophrenia. Increased interest in these receptors comes from animal and human studies that demonstrate antipsychotic potential of novel agonists of mGluR2/3 receptors and allosteric modulators of mGluR5. In this study, we have conducted a comprehensive examination of mGluR protein expression (mGluR1a, 2, 3, 4, 5 and 7) in human post mortem brain tissue to determine the regional specificity of distinct mGluR alterations in schizophrenia.

Methods: Human brain tissue was obtained from our brain collection, the Dallas Brain Collection (DBC). The tissue cohort consisted of 12 pairs of schizophrenia subjects and normal controls matched as closely as possible for age, brain pH, postmortem interval, and RNA integrity number. mGluR- specific antibodies were used in immunoblotting experiments to determine their individual expression levels in frontal and striatal brain regions, specifically the dorsolateral prefrontal cortex (DLPFC), anterior cingulate (ACC), caudate nucleus (CN) and nucleus accumbens (NAc). To determine potential effects of chronic antipsychotic treatment, Sprague-Dawley rats were given a first-generation antipsychotic (haloperidol), second-generation antipsychotic (risperidone) or placebo ($n = 10$ each) via drinking water continuously for 6 months following which they were sacrificed and frontal cortex and CN processed for immunoblotting experiments.

Results: (1) In the schizophrenia DLPFC, we find increased expression of mGluR1a ($t = 2.11$, df_{22}, $p = 0.047$) and mGluR7 ($t = 2.57$, df_{18}, $p = 0.02$) along with a reduction of mGluR3 protein ($t = 2.49$, df_{22}, $p = 0.02$). (2) mGluR1a was also increased in the ACC ($t = 2.5$, df_{22}, $p = 0.02$). (3) Significantly increased expression of mGluR2 ($t = 2.65$, df_{22}, $p = 0.15$) and mGluR5 ($t = 3.72$, df_{22}, $p = 0.001$) were found in the CN with a strong trend towards an increased expression of mGluR3 ($t = 1.99$, df_{22}, $p = 0.059$) in schizophrenia. There were no differences in any of the mGluRs examined in the NAc. Chronic antipsychotic treated animal experiments show that risperidone is associated with a decrease in mGluR1a ($t = 2.85$, df_{17}, $p = 0.01$) and mGluR7 ($t = 2.57$, df_{18}, $p = 0.019$) expression in the frontal cortex. In the CN, haloperidol treatment led to an increase in mGluR2 ($t = 7.8$, df_{18}, $p < 0.001$) and mGluR5 ($t = 2.5$, df_{18}, $p = 0.02$) protein expression.

Discussion: We find a distinct regional pattern of mGluR alterations in schizophrenia with the majority of mGluR alterations localizing to the DLPFC and CN. Data from chronic antipsychotic treated rodents suggest that the mGluR changes observed in the rodent frontal cortex are opposite to those seen in schizophrenia post mortem samples. This indicates that the changes in mGluR1, mGluR3 and mGluR7 in the DLPFC are not secondary to antipsychotic drug treatment and could be disease-specific. In the CN, however, the increases in mGluR2 and mGluR5 in schizophrenia could be due to antipsychotic drug treatment. While this could be a confound, it is also possible that these CN changes are related to the therapeutic effects of antipsychotic drugs. The localization of the mGluR alterations to the DLPFC and CN could suggest a role for mGluRs in cognitive deficits in schizophrenia. These data add to the body of literature implicating mGluRs in the pathophysiology of schizophrenia and suggest potential roles for modulators of mGluR 1, 3 and 7 in schizophrenia therapeutics.

Disclosure: S. Ghose: None. **K. Gleason:** None. **B. Potts:** None. **C. Tamminga:** Part 1: International Congress on Schizophrenia Research - Organizer; Unpaid volunteer American Psychiatric Association - Deputy Editor >$10,000 Finnegan Henderson Farabow Garrett & Dunner, LLP – Expert Witness >$10,000, Part 2: Intracellular Therapies (ITI, Inc.) - Advisory Board, drug development >$10,000 PureTech Ventures- Ad Hoc Consultant <$10,000 Eli Lilly Pharmaceuticles – Ad Hoc Consultant < $ 10,000 Sunovion – Ad Hoc Consultant < $10,000 Astellas – Ad Hoc Consultant < $10,000 Merck – Ad Hoc Consultant < $10,000.

107. Expression of Dopamine Receptor D1 and D2 Transcripts in Brains of Patients with Schizophrenia and Mood Disorders

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Background: DRD2 and DRD1 (dopamine receptor genes) have been functionally and genetically linked to schizophrenia, action of antipsychotic drugs, and cognition. There are three DRD2 splice variants: D2 short (D2S), D2 long (D2L) and D2 longer. D2S and D2L are expressed predominantly at pre- and postsynaptic sites, respectively. D2 longer is not well characterized.

Methods: We studied the expression of the three DRD2 splice variants and DRD1 in the dorsolateral prefrontal cortex (DLPFC) and hippocampus in large cohorts of subjects ($n = 685$ and 464, respectively), including patients with schizophrenia ($n_{PFC} = 176$, $n_{Hippocampus} = 101$), mood disorders ($n_{PFC} = 199$, $n_{Hippocampus} = 93$) and non-psychiatric controls (14^{th} week of gestation to 85 years of age, $n_{PFC} = 310$, $n_{Hippocampus} = 270$) using quantitative RT-PCR. The same subjects were genotyped for >100 SNPs in the two gene regions using the Illumina 650K BeadChips and TaqMan 5′ exonuclease allelic discrimination assay. A step-wise analysis was used for model selection for each transcript. Multiple linear regression was then used to examine the effects of diagnosis on mRNA expression. Furthermore we tested the relationship between expression and various measures of psychotropic medication (doses of antipsychotics expressed as CPZ equivalents, toxicology results, history of medication) within the patient groups. For SNP association analysis, SNP term was added to the selected model to obtain the association p value.

Results: In the DLPFC, D2S mRNA expression was significantly increased in schizophrenic patients relative to controls ($p < 10e\text{-}09$), while expression of D2L, DRD1 and D2Longer was significantly decreased in schizophrenia ($p < 10e\text{-}06$). Expression of the transcripts in patients with mood disorders showed an opposite pattern: there was reduced expression of D2S ($p < 0.03$) and increased expression of D2L and D1 ($p < 0.01$) in patients vs controls. The differences in the expression between patients and controls in the hippocampus were less pronounced than in the DLPFC: D2S expression was decreased in schizophrenics ($p < 0.05$), whereas the expression of D2L, D2longer and DRD1 did not differ from controls in hippocampus. Patients with bipolar disorder had increased expression of D2L, D2Longer and D1 ($p < 0.05$), while MDD patients did not differ significantly from controls. We did not detect significant relationships between psychotropic drugs' indices and expression for any of the transcripts. We have identified several significant associations between expression and SPNs. In agreement with the previous study (Bertolino *et al*., 2009; Glatt *et al*., 2009), the schizophrenia-associated risk allele of the intronic DRD2 SNP, rs2283265, was associated with an increased D2S/D2L ratio ($p < 0.05$) in the PFC, but not hippocampus, of control individuals.

Discussion: Although we cannot preclude the effects of medication on the expression of dopamine receptor transcripts, our data suggest that altered splicing of the D2 receptor and expression of D1 receptor may constitute a pathophysiological mechanism of schizophrenia and mood disorders. The association between schizophrenia risk-associated polymorphism and the ratio of D2S/D2L is consistent with this possibility. Our data also show that diagnostic differences are more pronounced in the DLPFC

than in the hippocampus, which may reflect either the technical issues (as the abundance of dopamine receptors is higher in the DLPFC than the hippocampus) or the neurobiology of the disease.
Disclosure: S. Kaalund: None. **E. Newburn:** None. **T. Ye:** None. **L. Wang:** None. **R. Tao:** None. **C. Li:** None. **R. Vakkalanka:** None. **A. Deep-Soboslay:** None. **L. Bigelow:** None. **R. Straub:** None. **T. Hyde:** None. **D. Weinberger:** None. **B. Lipska:** None. **J. Kleinman:** None.

108. Familial Abnormalities of Endocannabinoid Functioning in Schizophrenia and Bipolar Disorder

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Background: Recent research has provided epidemiological and experimental evidence pointing to the human endocannabinoid system's role in the pathophysiology of schizophrenia. First, and foremost, cannabis use is associated with a twofold increase in incidence of schizophrenia. Furthermore, the major psychoactive phytocannabinoid, delta-9-tetrahydrocannabinol, has been shown to induce psychotic symptoms in both healthy volunteers and schizophrenic patients. Recent work, however, has been able to demonstrate that the endocannabinoid anandamide in cerebrospinal fluid is not only elevated in acute schizophrenia but also in its initial prodromal states. As there is an inverse correlation between cerebrospinal anandamide levels and psychopathology, it can be hypothesized that endocannabinoid anandamide is associated with an adaptive if not protective mechanism countering neurotransmitter abnormalities in psychosis. Therefore, the endocannabinoid system could also be linked to vulnerability, and possibly heritable risk, for schizophrenia.
Methods: Within the framework of the present study we investigated levels of the eicosanoids anandamide, 2-arachidonoylglycerol, palmitoylethanolamide and oleoylethanolamide in plasma by liquid chromatography/tandem mass spectroscopy. In all we assessed plasma levels in a total of 39 twin pairs, with 25 twin pairs discordant for schizophrenia, 6 twin pairs discordant for bipolar disorder as well as 8 healthy pairs of twins.
Results: Both siblings of twin pairs discordant for schizophrenia showed significantly higher levels of anandamide and palmitoylethanolamide in plasma when compared to healthy twins ($p < 0.002$). The same held true for twin pairs discordant for bipolar disorder. We found no significant difference of any investigated eicosanoid in either groups of discordant twins. However, 5 initially healthy, discordant twins of schizophrenic patients as well as 3 initially healthy discordant twins of bipolar patients developed a psychotic disorder within five years after the initial investigation. Interestingly, in the group discordant for schizophrenia all initially healthy twins who later developed a psychotic disorder showed significantly lower levels of anandamide ($p = 0.042$) as well as 2-arachidonoylglycerol ($p = 0.049$) when compared to the discordant twins who did not develop any symptoms.
Discussion: Overall, we found significant elevations of endocannabinoid system components in serum related to familial vulnerability, but not to clinical state. The directionality of the effect agrees with a model indicating a protective role of anandamide in schizophrenia. Our results suggest an investigation of abnormal endocannabinoid signaling as a potential intermediate phenotype of schizophrenia.
Disclosure: D. Koethe: Part 1: Speaker's Board AstraZeneca Germany, Part 2: None other than employer. **F. Pahlisch:** None. **M. Hellmich:** None. **C. Rohleder:** None. **A. Meyer-Lindenberg:** None. **D. Piomelli:** None. **F. Leweke:** Part 1: Speaker's Bureau and/or Consultant: AstraZeneca GmbH Bristol-Meyers Squibb GmbH Lundbeck Germany Servier Germany CEO: curantis UG Ltd.

109. Subcellular Deficits of Glutamate Transporter Expression in Schizophrenia

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Background: While the treatment of schizophrenia with antipsychotic medications revolutionized the clinical management of this illness, approximately one-third of patients with schizophrenia have persistent positive symptoms despite multiple trials of antipsychotic medicines. Recently, new strategies for the treatment of schizophrenia have emerged, including modulation of glutamate receptors, an approach which was developed, in part, based on an accumulating body of evidence of alterations in glutamate transmission from postmortem, imaging, and preclinical studies. While the initial glutamate hypothesis of schizophrenia was focused on NMDA receptor dysfunction, this hypothesis has been extended to include other glutamate receptors, transporters, and enzymes involved in glutamate transmission. Postmortem findings of changes in the expression of glutamatergic molecules in schizophrenia may be conceptualized as functional alterations of remodeled glutamate synapses, secondary to the underlying pathophysiology of chronic severe mental illness and a lifetime of treatment with psychotropic medications. We have found decreased expression of glial glutamate transporters in subjects with schizophrenia, suggesting that glutamate synapses have alterations in glutamate buffering and reuptake capacity. Glutamate transporters facilitate excitatory neurotransmission by limiting glutamate spillover to adjacent synapses, and we postulate that the localization of excitatory amino acid transporters (EAATs) is altered in corticothalamic circuits in schizophrenia, contributing to the psychopathology of this disease. Specifically, we hypothesize that there are defects in the subcellular localization of EAATs in this illness.
Methods: To evaluate this hypothesis, we assessed the localization of EAAT isoforms using subcellular fractionization and Western blot analysis in postmortem tissue from subjects with schizophrenia and a comparison group. We also used immunofluorescence to identify the cell types expressing EAAT2 in schizophrenia.
Results: We detected expression of EAAT2 in both neurons and astrocytes in the prefrontal cortex schizophrenia. In the anterior cingulate cortex, we found an increase in the EAAT2B isoform of EAAT2 in a fraction containing extrasynaptic membranes in subjects with schizophrenia. EAAT2B protein levels were not changed in the ER or PSD containing fractions. We did not find any changes in the expression of EAAT1 or EAAT3 protein in any of the subcellular fractions.
Discussion: These data indicate that expression and localization of glutamate transporters is abnormal in schizophrenia, suggesting decreased perisynaptic buffering and reuptake of glutamate and increased glutamate spillover. These studies extend the glutamate hypothesis of schizophrenia beyond the NMDA receptor and provide new substrates for diagnosis and treatment of this often devastating illness.
Disclosure: R. McCullumsmith: None. **D. Shan:** None. **V. Haroutunian:** None. **J. Meador-Woodruff:** Part 2: Dr. Meador-Woodruff is paid to be the editor of Neuropsychopharmacology.

110. Stress-Induced Dopamine (DA) Response in Subjects at Clinical High Risk (CHR) for Schizophrenia with Concurrent Cannabis Use

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Background: While genetic and environmental factor predispose individuals to schizophrenia (SCZ), the actual onset of the disease has often been explained in the framework of stress and

neurochemical vulnerability of the dopaminergic (DAergic) system. Our recent neuroimaging data suggest heightened DAergic response to stress in both patients with SCZ and in individuals at clinical high risk (CHR), a finding consistent with the reported elevated amphetamine-stimulated DA release in SCZ, interpreted as DAergic *sensitization*. Cannabis is believed to contribute to the development of SCZ in a process that is speculated to involve DA. The present study investigates whether cannabis use (a risk factor for development of scz) in CHR is associated with increased DAergic response to stress as compared to CHR with no cannabis use.

Methods: 12 CHR (23 ±1.34 years) and 12 CHR who concomitantly used cannabis (CHR-CU, 24.25 ±1.38 years) matched for age and sex were included. CHR-CU were requested to abstain from using cannabis on the day of the PET scan. Subjects were scanned during a sensorimotor control task (SMCT) and under the stress condition using the validated Montreal Imaging Stress task (MIST). The SMCT and the stress scans were done ~ 7 days apart. The simplified reference tissue model (SRTM) was used to obtain BP_{ND} in striatal subdivision including limbic striatum (LST), associative striatum (AST) and sensorimotor striatum (SMST). Stress-induced DA release (indexed as a percent reduction in [^{11}C]-(+)-PHNO BP_{ND}) between CHR and CHR-CU was tested with ANOVA.

Results: BP_{ND} during the SMCT was not significantly different between groups in any brain region ($p > 0.21$). Stress-induced displacement was significantly smaller in CHR-CU relative to CHR in all regions investigated: AST (% $p < 0.001$), LST (% $p = 0.007$), SMST (% $p = 0.003$), GP (% $p = 0.09$), SN (% $p = 0.04$) and whole striatum (% $p = 0.001$); suggesting a blunting of the DA stress induced response in CU. There was a trend-like relationship between age of onset of cannabis use and displacement (AST: $r = 0.56$ $p = 0.09$) such that greater blunting was associated with early age of onset. There was a significant correlation ($r = 0.60$ $p = 0.05$) between the change in the *expectancy* to use marijuana following stress task (Marijuana Craving Questionnaire compulsivity scores, Heishman 2009) and stress induced displacement in the AST. All subjects experienced an increase in positive attenuated psychotic symptoms ($p = 0.001$) following the MIST.

Discussion: Our preliminary results suggest a blunted DA stress reactivity in short-term abstinent CHR who concurrently use cannabis, despite increase in attenuated positive psychotic symptoms. This finding does not appear to support the (cross)-sensitization hypothesis which posits greater DAergic reactivity to stress in cannabis users; however it adds to the growing body of literature showing blunted DA response to a DA elevating challenge in addiction. It also questions the mechanisms by which cannabis may increase the risk for schizophrenia.

Disclosure: R. Mizrahi: None. **I. Suridjan:** None. **I. Boileau:** None. **P. Rusjan:** None. **T. George:** Part 1: consult to Pfizer and Novartis and receiving income from them from consulting and speaker's fees of <$10,000 per year. Not greater than 5% of my total income. **A. Wilson:** None. **S. Houle:** None.

111. Deficits in Transcriptional Regulators of Cortical Interneuron Subpopulations in Schizophrenia

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Background: In schizophrenia, alterations within the prefrontal cortical (PFC) GABA system, such as lower mRNA levels for the GABA synthesizing enzyme GAD67, are most prominent in neuron subpopulations that contain the calcium-binding protein parvalbumin or the neuropeptide somatostatin. The transcription factors Lhx6 and Sox6 are selectively expressed by parvalbumin and somatostatin neurons in adult cortex, and germline deletion of Lhx6 or Sox6 profoundly reduces the number of cortical neurons immunoreactive for parvalbumin or somatostatin. Therefore, we sought to determine whether deficient expression of Lhx6 and/or Sox6 may contribute to the dysfunction of these GABA neuron subpopulations in schizophrenia.

Methods: We used a combination of quantitative PCR (qPCR) and in situ hybridization to measure mRNA levels in PFC area 9 from 42 schizophrenia subjects, each matched to one healthy comparison subject for sex and age. The mean age, postmortem interval, brain pH, RNA integrity number, and tissue storage time did not differ between subject groups. qPCR was performed using the comparative threshold cycle method with four replicate measures per target gene, and target gene expression levels were normalized using three reference genes. In situ hybridization was performed with an S^{35}-labeled antisense riboprobe for Lhx6 mRNA in 3 tissue sections from the first 22 subject pairs with available tissue sections, and optical density was measured in the gray matter of PFC area 9 and in individual cortical layers using film autoradiographs. An analysis of covariance model with subject pair as a blocking factor was used to test the effect of diagnosis on expression level for each target mRNA and to evaluate potential effects of storage time, brain pH, and RNA integrity number on mRNA levels. We also conducted similar studies in the PFC of monkeys chronically exposed to haloperidol, olanzapine, or placebo ($n = 6$ per treatment condition) for at least 17 months.

Results: Using qPCR, we replicated prior findings of lower mRNA levels for GAD67 (-14%), parvalbumin (-22%) and somatostatin (-36%) in a new cohort of schizophrenia subjects, and more severe deficits in these markers were consistently found in the same schizophrenia subjects. We also found schizophrenia-related deficits in Lhx6 mRNA levels that were more pronounced (-23%) in the same schizophrenia subjects who had lower GAD67, parvalbumin, and somatostatin mRNA levels. Furthermore, Lhx6 mRNA levels were correlated with mRNA levels for GAD67, parvalbumin, and somatostatin across individual subjects. Using in situ hybridization, we also found that Lhx6 mRNA levels were significantly lower in cortical layers 3, 5, 6, and almost in layer 2, suggesting that lower Lhx6 mRNA levels are present in the same cortical layers where PV neurons (layers 3-4) and SST neurons (layers 2, 5, and 6) are most commonly found. In contrast, Sox6 mRNA levels were unchanged in schizophrenia. Finally, analyses of subsets of schizophrenia subjects and antipsychotic-exposed monkeys suggested that lower Lhx6 mRNA levels in schizophrenia were not attributable to psychotropic medications or illness chronicity.

Discussion: These data suggest that substantial deficits in multiple GABA neuron-related markers generally cluster together in the same schizophrenia subjects, and that deficient Lhx6 mRNA expression may contribute to the dysfunction of specific GABA neuron subpopulations in these schizophrenia subjects.

Disclosure: D. Volk: None. **T. Matsubara:** None. **T. Hashimoto:** None. **E. Sengupta:** None. **D. Lewis:** Part 1: Dr. Lewis has served as a consultant in the areas of target identification and validation and new compound development to BioLine RX, Bristol-Myers Squibb, Merck, and SK Life Science., Part 4: Dr. Lewis currently receives investigator-initiated research support from the BMS Foundation, Bristol-Myers Squibb, Curridium Ltd and Pfizer.

112. Diminished In-Vivo Gamma-Aminobutyric Acid Concentrations in the Visual and Prefrontal Cortices in Recent Onset Schizophrenia and Correlations with Gamma Band Power

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Background: The gamma-aminobutyric acid (GABA) hypothesis of schizophrenia proposes that reduced neuronal GABA concentra-

tion and neurotransmission results in cognitive impairments in schizophrenia. Few in-vivo studies have directly examined this hypothesis. Among the published studies, there has been a notable lack of consistent evidence for the predicted reduction in GABA concentration in schizophrenia. Therefore, the replication of GABA MRS results is needed to demonstrate the reliability of this measure. Additionally, to our knowledge, no published study has examined whether there is reduced GABA in the dorsolateral prefrontal cortex (DLPFC) in schizophrenia. With this study, we sought to replicate in an independent sample of recent onset subjects our prior finding of reduced GABA concentrations in the visual cortex. We also sought to obtain preliminary evidence of a reduction in GABA levels in the DLPFC in this sample. Additionally, the availability of EEG data among a subset of subjects with DLPFC GABA measurements provided an opportunity to determine whether these measures were correlated with levels of gamma band power, as suggested by models of GABAergic regulation of high frequency oscillations in prefrontal neurons during cognition.

Methods: We scanned 7 subjects with recent onset schizophrenia (within three years of diagnosis) and 9 demographically matched healthy control subjects on a Siemens 3T Trio Tims MRI system. To obtain GABA spectra, we employed proton magnetic resonance spectroscopy (MRS) using a spin-echo variant of a J-difference editing pulse sequence, developed by Siemens, essentially identical to the MEGA-PRESS sequence. For the visual cortex, a $30 \times 25 \times 25$ mm volume of interest was centered over the calcarine sulci bilaterally. For the DLPFC, we placed a $30 \times 15 \times 35$ mm voxel within the anatomical boundaries of left middle frontal gyrus. With jMURI we quantified GABA by estimating the area underneath the GABA peak and normalizing this value with that of the creatine peak. This GABA/Cr ratio was used for hypothesis testing. Given the significant potential impact of in-scanner movement on our measurements and between group comparisons, we employed a novel quantitative head movement tracking procedure to ensure that all subjects experienced minimal in-scanner movement. EEG data were acquired while subjects completed the POP task, a paradigm that manipulates the amount of cognitive control required across conditions. EEG was completed using Neuroscan 128-electrode Quick-Cap and Neuroscan SynAmps hardware. Preprocessing and analysis was completed in EEGLab and included 0.5Hz high-pass filtering and blink artifact subtraction using ICA. Gamma band (30-80 Hz) power was measured using a complex Morlet wavelet, referenced -200 to oms pre-cue. Measurements (in decibels) were calculated for the delay period of the POP task with electrodes corresponding to the left frontal regions.

Results: In the visual cortex we found an $\sim$11% reduction in GABA concentration in the schizophrenia (SZ) group compared to the control (C) group, $P = .06$, one-tailed t-test, effect size $= .76$. In the DLPFC, we found an $\sim$8% reduction in the SZ group, $P = .28$, two-tailed t-test, effect size $= .60$. Among 9 subjects with both MRS and EEG data, we found a correlation of $r = .42$ between the DLPFC GABA concentration and gamma band power during the delay period of the high cognitive control condition. Among SZ subjects ($n = 7$), this correlation was .74. All subjects displayed minimal head movement during MRS scanning and there were no group differences in movement.

Discussion: We found preliminary evidence of reduced in-vivo GABA concentrations in the visual cortex in a cohort of subjects with recent onset schizophrenia. If sustained and statistically confirmed in a larger sample, this ongoing study will provide a replication of our prior results, suggesting that reduced visual cortical GABA can be reliably measured and may be a robust illness biomarker. We also found preliminary support for a reduction in GABA levels within the DLPFC. As predicted, we also found preliminary evidence for correlations between gamma band power and GABA concentrations from the DLPFC. These results significantly extend the diverse lines of evidence supporting the centrality of DLPFC dysfunction in the pathophysiology of cognitive deficits in schizophrenia. The fact that these results were obtained in a sample of recent onset patients suggest that illness chronicity is likely not a major confound. Our movement tracking findings suggest that in-scanner movement does not confound our results.

Disclosure: J. Yoon: None. **R. Maddock:** None. **M. Minzenberg:** None. **M. Buonocore:** None. **R. Ooms:** None. **G. Gomes:** None. **C. Carter:** Part 1: Provided one time consultation to Pfizer, Merck, Lilly, and Servier.

113. Central Pathways of Pain and Pleasure Interact

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Background: Observing another individual in pain elicits a predictable network of neural activation. This network, however, is modulated by several physiological and psychological factors related to the perspective being taken. For example, a participant may be instructed to take the perspective of a person not being injured or to take the perspective that the injury is a medical treatment necessary for the recipient's recovery from illness. Moreover, reliable hemodynamic activation in a network of brain regions has been observed in response to pleasurable or painful stimuli.Shared activations, for both pain and pleasure, have been found in the amygdala, insula, anterior cingulate cortex (ACC), and striatum. The current study applied functional neuroimaging (fMRI), self-report of subjective appetitiveness and aversiveness of stimuli, and autonomic measures to explore the neural pathways common to both pain and pleasure.

Methods: A representative sample of 60 males (18-30 years of age) was tested. Participants were selected based on whether they enjoyed watching sports in which the explicit aim is to injure another person (e.g.,boxing, mixed martial arts, etc), or found such activities abhorrent. While in the fMRI scanner, subjects were shown 20 10-second videos of violent sports or control conditions of similar content containing no injuries or intent to injure. Data collected included fMRI, autonomic activity (heart rate variability, electrodermal activity, respiration), salivary cortisol, and testosterone. These measures were taken before or during the observation of videos. In addition the short version of the Big 5 personality traits and indexes of psychopathy and alexithymia were assessed.

Results: Relative to a control condition (i.e., similar visual content but no intent to injure) increased neuro-hemodynamic activation was observed in the subgenual region of the ACC, the right amygdala, pre-motor cortex, primary motor cortex and bilaterally in the insula. Higher scores on an index of psychopathy were correlated with greater bilateral activation in the head of the caudate nucleus. Higher scores on an index of alexithymia were correlated with increased prefrontal activation as well as ACC activation, and were inversely correlated with activation of the anterior medial cingulate cortex.

Discussion: These data suggest that brain regions previously implicated in representations of pain and pleasure are simultaneously recruited in individuals who report enjoyment when watching violent sports. Relations were observed between activation of the neural networks and features of alexithymia and psychopathy. These data have implications for understanding the relationship between personality traits and the brain-behavior mechanisms involved in pleasure and pain. In addition, knowledge of shared pathways involved in these behaviors can inform our understanding of mechanisms underlying mental health conditions such as antisocial personality disorder, psychopathy, and sadism.

Disclosure: E. Porges: None. **J. Decety:** None.

114. A Multi-Modal Investigation of Treatment Response Associated with Electroconvulsive Therapy in Major Depressive Disorder

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Background: A thorough understanding of the neural correlates associated with treatment response with ECT will have profound impact on safer, less-invasive neuromodulation treatments for major depression. Previous longitudinal studies with positron emission tomography (PET) and single photon emission computed tomography (SPECT) have had discrepant results showing both increased and decreased cerebral metabolism. We propose to use the superior temporal resolution of magnetoencephalography (MEG) and the superior spatial resolution of functional magnetic resonance imaging (fMRI) to further elucidate the neural correlates of treatment response in major depressive disorder. This will be the first longitudinal study of ECT with fMRI and MEG. For this abstract, we used functional magnetic resonance imaging (fMRI) and independent component analysis (ICA) to assess changes in the temporal dependencies between independent neural components (functional network connectivity) associated with electroconvulsive therapy.
Methods: Ten patients with major depressive disorder (mean age: 67.4 years; range: 50 to 82 years) completed at least one scan and assessment of study protocol. Depression severity was assessed with the Hamilton Rating Scale for Depression – 24 item (HRSD-24), Thase Core Endogenomorphic Scale, and the Brief Psychiatric Rating Scale (BPRS). Subjects received between 8 and 19 treatments of twice or thrice weekly bitemporal or right unilateral electroconvulsive therapy (stimulus delivery determined by titration of seizure threshold). Clinical response determined the number of treatments. All subjects were concurrently treated with antidepressant medications. Neuropsychological assessment was completed with the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Trails A and B, CLOX I and II, and the Grooved Pegboard Test. The post-scan imaging data was completed $\geqslant$5 days after the last ECT treatment to mitigate the immediate effects of seizure activity on the imaging data. Functional magnetic resonance imaging parameters were as follows: FOV 24 cm, matrix size 64x64, TR/TE = 2000/30 msec, and slice thickness 3.5 mm. FMRI data was preprocessed with SPM5 and INRIalign. Group ICA fMRI Toolbox (http://icatb.sourceforge.net) analyzed the preprocessed data with higher model order (second data reduction step with 75 components). The Functional Network Connectivity Toolbox assessed the correlations between 16 non-artifactual independent components with a lag correlation.
Results: At the present time, five subjects completed the fMRI study protocol before and after the ECT series. These subjects had a reduction in symptom severity as measured by the HRSD-24 ($t = 3.8$, $p = 0.03$) and Thase Core Endogenomorphic Scale ($t = 6.1$, $p = 0.009$). Despite the complaint of cognitive impairment in two of the five subjects, changes in neuropsychological functioning were not significant ($p \geqslant 0.15$ on all cognitive domains of the RBANS, Trails, and Grooved Pegboard). Functional network connectivity revealed significant (uncorrected) changes between two pairs of independent components (named after anatomic area associated with maximal t-score): component 8 (lingual gyrus, Brodmann Areas 18, 19) and component 46 (superior temporal gyrus, BA 12, 21) ($p = 0.02$); component 4 (culmen) and component 47 (inferior frontal gyrus, BA 47) ($p = 0.003$). The between network correlations were negative or weakly correlated prior to ECT and positively correlated after ECT. A qualitative review of long-term follow-up ($>$ 1 month after last treatment) indicated that a sustained treatment response appeared to be associated with the degree of change between networks.
Discussion: These preliminary results are presented in the context of ongoing patient recruitment of patients with major depression receiving electroconvulsive therapy. The current, preliminary results suggest that treatment response during electroconvulsive therapy is associated with increased temporal dependencies between specific pairs of networks in individuals with major depression. Future work will include in age-matched sample of healthy comparisons to assess whether these changes between networks are associated with normalization of temporal dependencies. Furthermore, this study will utilize the superior temporal resolution of magnetoencephalography to elucidate the neuropathological correlates of treatment resistant depression and electroconvulsive therapy treatment response.
Disclosure: C. Abbott: None. **R. Thoma:** None. **K. DePlonty:** None. **J. Lewine:** None. **J. Turner:** None.

115. Cross-Sectional and Longitudinal Abnormalities in Brain Structure in Children with Severe Mood Dysregulation or Bipolar Disorder

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Background: Debate centers on whether severe chronic irritability is a developmental presentation of bipolar disorder (BD). Leibenluft *et al.* operationalized severe mood dysregulation (SMD) to capture children with chronic, non-episodic irritability. Research has demonstrated structural brain abnormalities in BD; however, no study has compared brain anatomy in children with SMD to those with BD and healthy volunteers (HV). In addition, although research shows that structural abnormalities in pediatric BD are present early in the disease course, the developmental trajectory of these abnormalities is unclear. This study reports on structural imaging data in children with BD and SMD compared to HV, including both cross-sectional and longitudinal data in these three groups.
Methods: T1-weighted structural scans from 209 children (86 with SMD, 55 with BD, and 68 HV) were acquired on a 1.5-T MRI scanner. An optimized, modulated voxel-based morphometry (VBM) analysis was conducted using the VBM2 package in SPM2. After processing, a whole-brain F-test in SPM2 was used to compare SMD, BD and HV. In addition, 31 SMD, 34 BD, and 28 HV were scanned a second time (average time between scans 1.99 $\pm$ 0.94 years), and both scans for each subject were processed using the longitudinal data utility in the VBM2 package. Then, a whole-brain F-test in SPM2 was used to examine the interaction between group (SMD vs. BD vs. HV) and scan time (first vs. second scan). In both analyses, clusters that surpassed a height threshold of $p < 0.001$ uncorrected and an extent threshold of 150 voxels were identified as significant. Mean VBM values in significant clusters, representing the gray matter probabilities across the cluster, were calculated for each subject at each time point. Within- and between-group post-hoc tests were run on these mean cluster values in SPSS.
Results: *Cross-sectional Analysis*: There were between-group differences in gray matter volume in several clusters, including in bilateral precuneus and superior parietal lobule, bilateral superior and medial frontal gyri, and right insula. Post-hoc analyses indicated that the between-group differences in these clusters were driven mainly by reduced gray matter volume in the BD group, as compared to the SMD and HV groups. *Longitudinal Analysis:* Group $\times$ time interactions were evident in the right precuneus/cuneus and superior parietal lobule, right caudate, right anterior cingulate cortex (ACC), and left superior frontal gyrus. Post-hoc decompositions revealed that these interactions were

driven by an abnormal developmental pattern in BD in all cases, either an absence of normal changes or an inverse pattern of development. In most regions, the longitudinal pattern did not differ between SMD and HV.
Discussion: These data suggest pediatric BD is associated with reduced gray matter volume in parietal and frontal regions, and in the insula. In addition, children with BD, but not those with SMD, exhibit an abnormal developmental trajectory in several of these regions, as well as in the caudate and anterior cingulate gyrus. The longitudinal findings are predominately in frontal and parietal regions, in addition to the ACC and caudate, and are largely driven by the lack of normal volumetric change patterns in BD that are evident in the HV and SMD groups over time. Other VBM studies in patients with BD have described abnormalities in similar regions, however, the direction of these abnormalities has not been clear; this diversity has been hypothesized to be caused by medication effects on gray matter. A VBM study of unaffected first-degree adult relatives of patients with BD found that genetic risk for BD was specifically associated with gray matter deficits in the R ACC and ventral striatum (McDonald *et al*., 2004). Thus, our findings of developmental abnormalities in youth with BD suggest that structural deficits in these regions may be core features of the illness that are present in both adults and youth with BD, as well as in unaffected relatives. It is especially noteworthy that children with SMD, in contrast to those with BD, displayed relatively normal gray matter volume and structural development in the present study. Our findings give further support to the evidence suggesting that SMD is not a developmental phenotype of BD, but a distinct disease process with a different etiology. It also raises the possibility that, compared to BD, the symptomatology of SMD is based more in dysfunctional neural circuitry than in structural abnormalities.
Disclosure: N. Adleman: None. **V. Razdan:** None. **S. Fromm:** None. **R. Kayser:** None. **D. Dickstein:** None. **D. Pine:** None. **E. Leibenluft:** None.

116. Gray Matter Alterations in Type 2 Diabetes and Major Depression Assessed by Voxel-Based Morphometry

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Background: Type 2 diabetes is a major public health problem characterized by numerous complications including renal disease, retinal disease and peripheral neuropathy. The purpose of this study was to examine the effect of type 2 diabetes and major depression on gray matter using voxel-based morphometry (VBM). We hypothesized that diabetic subjects and depressed subjects will demonstrate decreased gray matter in prefrontal areas as compared to healthy control subjects. We also hypothesize that diabetes and depression combined will demonstrate greater effects than either disease alone.
Methods: Subjects with type 2 diabetes ($n=18$), subjects with major depression ($n=50$), and subjects with both type 2 diabetes and major depression ($n=27$) were compared to healthy controls ($n=44$). Gray matter volume differences were assessed using FSL-VBM. Data was analyzed using a 2x2 ANCOVA, assessing the effect of diabetes, depression, and the interaction effect controlling for age, gender, and education. Gray matter changes were also correlated with clinical measures such as depression severity measured by the Center for Epidemiological Studies Depression scale and diabetes severity measured by hemoglobin A1c levels (Hgb A1c).
Results: There was a significant diabetes effect with evidence of gray matter loss in the right anterior cingulate, right superior temporal gyrus, right inferior frontal gyrus, and the left insula. Regions of gray matter loss associated with depression or the interaction of diabetes and depression did not survive correction for multiple comparisons. Gray matter volume losses in similar regions affected by diabetes correlated with Hgb A1c. There were no significant correlations with depression severity.
Discussion: In previous work published by our group, we have demonstrated gray matter volume loss in the anterior cingulate and orbitofrontal cortex in diabetic subjects with and without depression. Using a different method in a different subject population, we have replicated findings of lower gray matter volume in the medial prefrontal cortex associated with type 2 diabetes. The lack of a significant depression effect or interaction effect was surprising. It may be that specific subtypes of depression may be more associated with gray matter volume losses. The neuroanatomical sequelae of type 2 diabetes detected by VBM may underlie cognitive impairments associated with the disease.
Disclosure: O. Ajilore: None. **A. Zhang:** None. **S. Yang:** None. **A. Kumar:** None.

117. Amygdala and Prefrontal Dysfunction during Face processing in Pediatric and Adult Patients with Bipolar Disorder: Role of Face Emotion and Attentional Demands

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Background: Research in bipolar disorder (BD) implicates cortico-limbic dysfunction during face emotion processing; however, abnormalities vary across studies. In particular, it remains unclear which attention conditions (i.e., explicit, in which attention is focused towards face emotion, or implicit, in which attention is not), and which face emotions (angry, happy, fearful, and/or neutral) elicit dysfunction in the amygdala and prefrontal cortex (PFC). Moreover, such dysfunction has not been examined developmentally by comparing pediatric vs. adult patients, despite the fact that both groups demonstrate face emotion labeling deficits. It is difficult to determine the impact of specific face emotions and attentional demands on neural perturbations in BD, in part, because studies have yet to include multiple face emotions and attention states in one analysis. Here, we report data from a relatively large sample (62 pediatric and adult patients and 119 healthy comparison subjects), in which we compared neural activity between patients and healthy comparison subjects across different attention conditions and face emotions while using a paradigm that was brief enough to be tolerated by affected children as well as adults.
Methods: Functional magnetic resonance imaging (fMRI) data were acquired from 181 participants, including 62 BD patients ($N=36$ children; $N=26$ adults) and 119 healthy comparison subjects ($N=57$ children; $N=62$ adults). Subjects attended to implicit (i.e., nose width, passive viewing) and explicit (i.e., hostility, subjective fear) aspects of emotional (i.e., angry, happy, fearful) and none-motional (i.e., neutral) faces. We conducted a whole-brain analysis in Statistical Parametric Mapping 8 (SPM8), using a statistical threshold of $p<0.001$, uncorrected, and a voxel-wise extent threshold of $k=20$. The primary analysis consisted of a four-way ANOVA [2 (age group: child, adult) $\times$ 2 (diagnosis: BD, healthy comparison) $\times$ 4 (face emotion: angry, happy, fearful, neutral) $\times$ 4 (attention state: fear, hostility, nose width, passive viewing)]. Since there were no suprathreshold voxels in the four-way ANOVA, analyses focused on the significant three-way [2 (diagnosis) $\times$ 4 (face emotion) $\times$ 4 (attention state)] interactions.
Results: Behaviorally, pediatric BD patients rated neutral faces as more hostile than did all other groups (p's $<.01$). Whole brain

analyses revealed amygdala, ventral PFC (vPFC), and anterior cingulate dysfunction in BD relative to healthy comparison subjects. Both pediatric and adult BD patients demonstrated amygdala hyperactivation during explicit (i.e., hostility) ratings of fearful faces ($p<.01$), and implicit (i.e., passive) viewing of angry and neutral faces ($p's<.01$). Compared to healthy comparison subjects, BD patients demonstrated vPFC hyperactivation during passive viewing ($p<.01$), but vPFC hypoactivation when attending to subjective fear ($p<.01$) or when completing nose width ratings ($p<.01$) of angry faces. The pattern in the anterior cingulate was similar to that in the vPFC, i.e., when viewing angry faces, BD patients showed anterior cingulate hyperactivity during passive viewing ($p's<.01$), but hypoactivity while rating subjective fear ($p's<.01$). Within the BD group, there was no relationship between mood ratings (i.e., depression and mania scores) and amygdala, vPFC, or anterior cingulate activation ($p's>.14$).

Discussion: To our knowledge, this is the first whole-brain analysis including multiple face emotions and attention conditions in a large sample of adults and children with and without BD. Consistent with prior imaging findings and clinical features of the illness, we found amygdala, vPFC, and anterior cingulate dysfunction in both adults and children with BD. Taken together, these findings suggest pervasive amygdala dysfunction in BD while processing negatively-valenced and neutral faces, regardless of the attentional condition. In contrast, vPFC and anterior cingulate abnormalities were more sensitive to task-demands. Future imaging studies should continue to examine stimulus valence and attentional demands in the pathophysiology of pediatric and adult BD. Moreover, work is needed to explore the functional connectivity of frontal and limbic structures in the development and maintenance of the illness.

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118. Major Depressive Disorder and Bipolar Disorder Subjects exhibit Different Functional Brain Activation during a Depressive Episode

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Background: Bipolar disorder (BPD) is a serious psychiatric illness that affects approximately 1.5% of the U.S. population and represents a significant source of individual morbidity and societal cost. Patients with BPD spend considerably more time with depressive rather than manic symptoms and suffer greater morbidity during depression. However, little research has focused on understanding the neurophysiology of bipolar depression. Comparing brain activation in subjects with BPD during depression to healthy subjects and subjects with major depressive disorder (MDD) may provide neurophysiological correlates of depression associated with BPD. Prior neuroimaging studies in BPD suggest that there is potential disruption in cortico-limbic networks responsible for emotional homeostasis. With these considerations in mind, we examined depressed BPD and MDD subjects and healthy subjects with fMRI. In prior fMRI studies the anterior cingulate gyrus (ACC) exhibited altered activation in both MDD and BPD. Yet each disorder seems to show a unique disruption in cortico-limbic networks. Therefore, we hypothesized that a functional connectivity analysis using the ACC as the seed region would reveal a unique pattern of altered connectivity in each disorder. Specifically, we predicted that BPD subjects would show greater altered connectivity with ventral frontal regions compared to MDD.

Methods: Twenty-four patients with BPD, 21 patients with MDD, and 14 healthy subjects between ages 18 and 45 were recruited from the University of Cincinnati Academic Health Center. MDD and BPD subjects were recruited during a depressive, had no current substance use disorders, and were taking no psychiatric medication. All subjects were evaluated using the Structured Clinical Interview for DSM-IV and gave informed consent before participating. Subjects were scanned at the University Of Cincinnati College Of Medicine's Center for Imaging Research (CIR) using a 4.0 Tesla Varian Unity INOVA Whole Body MRI/MRS system (Varian Inc., Palo Alto, CA). During the fMRI task, subjects performed a Continuous Performance Task with Emotional and Neutral Distracters (CPT-END). The CPT-END task involves a visual oddball paradigm with the addition of emotional and neutral pictures taken from the International Affective Picture System (IAPS; University of Florida). A voxel-wise functional connectivity analysis was then performed using the right and left ACC as seed regions.

Results: There were no significant differences in race, age, or sex among groups. Healthy subjects had significantly more years of education than BPD ($F=2.5$, $p<.01$) and MDD ($F=2.8$, $p<.01$) subjects. There was no difference in education between the patient groups nor was there a difference in YMRS or HAM-D ratings. The functional connectivity analysis showed significantly increased correlation of the left ACC with the left medial frontal gyrus (Brodmann area 10) in MDD compared to BPD subjects. The right ACC showed significantly increased correlation with the insula (Brodmann area 13 and 44), left medial frontal gyrus (Brodmann area 10), left ACC (Brodmann area 32), and right superior temporal gyrus (Brodmann area 22) in BPD compared to MDD subjects. All reported regions were significant at $p<0.05$ corrected with a voxelwise threshold of 0.005 and a cluster threshold of 20 or more contiguous voxels which gives a false discovery rate of <0.05

Discussion: Consistent with our initial hypothesis, the ACC showed different patterns of connectivity in subjects with BPD and MDD. The medial frontal gyrus, involved in regulating emotion, showed opposite patterns of connectivity with the left and right ACC in the two depressed groups. This increased connectivity may represent compensatory cortical activity necessary to successfully complete the task. The right ACC showed increased connectivity with the insula in BPD compared to MDD subjects. The insula is involved in emotional appraisal and empathy and the altered connectivity in this region may be involved in generating excessive negative emotions in the BPD subjects. Together, these findings suggest the ACC may be part of distinct dysfunctional networks in the two disorders. These connectivity differences support our general premise that the two types of depression are caused by different neurophysiological mechanisms.

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University Other: Chaired symposium for Consensus Medical Communications (CME through U. Minnesota; unrestricted grant from Ortho McNeil/Janssen); Mentored young investigator meeting – American Psychiatric Association; Directed discussion on Web MD, Part 4: As primary investigator - Janssen; as co-investigator - Eli Lilly, Janssen/J&J, AstraZeneca, Sumatomo, Pfizer, Part 5: No.

119. Cortical Thickness in Medicated and Unmedicated Adolescents with Major Depressive Disorder

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Background: Major depressive disorder (MDD) frequently starts in adolescence, and is associated with a especially worse outcomes including suicide. There has been growing interest in addressing the problem early in the disease course, taking advantage of the malleability of neural systems prior to maturity, before detrimental mechanisms become entrenched. A recent neuroimaging study that examined multi-generational offspring of individuals with MDD has identified a candidate biomarker for MDD [1]. This study found cortical thinning in the MDD group, which was present in both affected (to a greater degree) and unaffected offspring (to a lesser degree.) Closer examination of cortical thickness in adolescent depression is warranted, since cortical thickness normally decreases during adolescence [2].
Methods: We examined 34 adolescents aged 12-20 (15 adolescents with MDD who were taking medications, 10 adolescents with MDD who were unmedicated, and 9 healthy controls). Clinical assessment included a diagnostic interview and rating scales such as the Beck Depression Inventory (BDI). Participants underwent scanning at the Center for Magnetic Resonance Research at the University of Minnesota on a 3T Siemens scanner. Parameters for 3D T1 MPRAGE scan included: TR = 2530ms, TE = 3.63ms, T1 = 1100ms, 1mm slices, FOV 256, 256x256, flip angel = 7 (10minutes). Cortical reconstruction and volumetric segmentationwas performed with Freesurfer software (http://surfer.nmr.mgh.harvard.edu/). Group analyses of cortical thickness were conducted using FreeSurfer's Qdec, which allows group comparison of thickness at each vertex by applying a general linear model (GLM). Spatial thickness distribution was smoothed with a circularly symmetric Gaussian kernel of 15 mm full width half maximum to provide normal distribution of results. We first compared the unmedicated MDD group to controls, using age as a covariate. Second, we compared the unmedicated MDD group to the medicated MDD group, again using age as a covariate. To explore whether cortical thickness is more related to age in one group than in the other, we selected the DODS (different onset, different slope) option for the design matrix, which assumes different offsets but a different impact of age in both diagnostic groups (different slope). All GLM results were corrected for multiple comparisons utilizing a pre-cached cluster-wise Monte Carlo simulation implemented in Qdec. To further investigate regions that were identified in these group analyses, we used the mean cortical thickness values generated by the predefined, automatically parcellated FreeSurfer regions and ran correlations with clinical data.
Results: The analysis comparing the unmedicated MDD group to controls revealed a large cluster in the right hemisphere, middle temporal gyrus, extending into the inferior and superior parts of the temporal lobe. This cluster indicated a significantly steeper decline in cortical thickness with age in the MDD group compared to the control group, which showed a much flatter decline with age. No clusters survived multiple comparisons correction in the left hemisphere for this comparison. The second analysis, comparing the unmedicated MDD versus the medicated MDD groups, revealed a cluster in the same areas of the right temporal lobe. This cluster indicated a significant difference in the association between thickness and age across groups: the medicated patients showed a flatter appearance (more like the controls) as opposed to the steep decline seen in the unmedicated patients. Examining all adolescents together, correlation analyses for middle temporal region revealed a significant negative correlation between thickness and severity as measured by the BDI total score ($r = -.396$, $p = 0.03$) and with 7 of the subscales on the BDI (sadness, pessimism, past failure, self-dislike, suicidal thoughts, loss of interest, and loss of energy.)
Discussion: These results confirm right-sided cortical thinning as a biomarker for MDD; in this sample, accelerated thinning was observed with age in the unmedicated adolescents with MDD in the middle temporal cortex, and thickness in this area was negatively associated with depression severity. Further, our results suggest that medication may serve to alter this trajectory, and slow the rate of thinning to a more normal trajectory. These cross-sectional results need confirmation in a longitudinal study.
References:
1. Peterson BS *et al.* Cortical thinning in persons at increased familial risk for major depression. *Proc Natl Acad Sci USA* 2009; **106**(15): 6273-8.
2. Raznahan A *et al.* How does your cortex grow? *J Neurosci* 2011; **31**(19): 7174-7.
Disclosure: K. Cullen: None.. **B. Klimes-Dougan:** None. **K. Lim:** None.

120. Measurement of Brain GABA Concentration by Magnetic Resonance Spectroscopy in Postpartum Depressed Women: A Feasibility Study of the Worcester Foundation for Biomedical Research

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Background: Women with mood disorders are at elevated risk of developing postpartum depression (PPD) after giving birth and remain at high risk of PPD recurrence with subsequent pregnancies. Differential regulation of neuroactive steroids (NAS) during the perinatal period in animals has been associated with the development of postpartum-syndrome through their interaction with ã-aminobutyric acid (GABA) receptors and the abrupt fall in NAS plasma levels associated with parturition may trigger PPD in at-risk women. Few studies have investigated the role of GABA and NAS in high risk women. We investigated the antenatal and postpartum levels of GABA and NAS in two cohorts of perinatal women and prospectively evaluated depression, anxiety, sleep, social support and quality of infant bonding. Postpartum depressed women and healthy postpartum women underwent ^{1}H magnetic resonance spectroscopy (MRS) to quantify GABA levels in the anterior cingulate (ACC) and occipital (OCC) cortices. Preliminary MRS data has been presented here.
Methods: A prospective observational cohort study evaluated 31 subjects of 18-40 yrs. of age during pregnancy (i.e. at 26-30 and 34-36 weeks gestation) and in the postpartum (i.e. <36 hours after parturition and 3-10 weeks). Two cohorts were studied: healthy control subjects (HCS) ($n = 4$; mean age: 30 ± 3.16) and those at high-risk for developing PPD ($n = 6$; mean age: 30.83 ± 5.56). Serial

plasma NAS and GABA measurements and mood and psychosocial assessments were completed at each of 4 study visits. High-risk subjects who developed unipolar PPD were examined cross-sectionally in comparison to the HCS postpartum cohort using ^{1}H MRS. MRS data was acquired with 3.0T Philips Achevia whole-body MR system using phased-array receiver SENSE Head coil (Philips Health-Care, the Netherlands). Diagnostic high resolution anatomical brain MRI was performed on all subjects to exclude the existence of any pathology and to be used for MRS voxel localizations. Edited MRS spectra were acquired using MEscher-GArwood Point-REsolved Spectroscopy Sequence (MEGA-PRESS) (TE = 68 msec and TR = 2000 msec). This frequency selective technique enabled us to detect the GABA peak of the spectra by eliminating the signal due to Creatine (Cr). Two MRS runs were performed with each subject: Voxels positioned at the ACC ($3.0 \times 3.0 \times 2.0$ cm^3) or at the OCC ($3.0 \times 3.0 \times 3.0$ cm^3) regions. The data processing and fitting were done by code written using Matlab (The Mathworks, MA, USA). The difference-edited GABA signal at 3 ppm and the Creatine peak from unsuppressed PRESS signal was fit with a Gaussian models ($r > 0.95$) and the area under these fits were calculated to have GABA to total creatine (GABA/tCr) ratios. We did two-sample t-test, with the assumption of equal variances, to compare GABA/Cr ratios between HCS and PPD patients. We performed Pearson's correlation to investigate the relationship between the GABA/Cr ratios and age.

Results: This ongoing feasibility study has limited power to detect significant differences due to the small sample sizes analyzed. In the OCC, the GABA/Cr ratios for the HCS are 2.4% higher than PPD subjects, but this difference is not significant (range of ratios- HCS: 0.12-0.16; PPD: 0.10-0.15). In the ACC, the GABA/Cr ratios are lower in both groups compared with the ratios in the OCC. Although the GABA/Cr ratios are 6.1% lower for the HCS compared with PPD subjects, this difference is not significant (range of ratios- HCS: 0.05-0.14; PPD: 0.08-0.11). In addition, there is no significant difference between the group populations in terms of age; however we observe a difference in the GABA/Cr trend as a function of age. For ACC, the GABA/Cr ratios increase with age in HCS ($r = 0.91$, $p = 0.09$). We do not observe this trend for PPD subjects ($r = -0.42$, $p = 0.40$). Additional data on grey and white matter segmentation within the MRS voxels, to ensure the same contributions for each group, will be presented.

Discussion: There are no significant differences in GABA/Cr ratios in the OCC and ACC between healthy postpartum and postpartum depressed women. There is a trend for GABA/Cr ratios to increase with age in HCS compared to no change with age in PPD subjects. If this is replicated in a larger sample, then age may be an important consideration in further MRS investigations. Given the pilot nature of this feasibility study, further studies will need larger sample sizes to detect potential differences in GABA/Cr ratios among our cohorts. Analysis of plasma GABA and NAS is underway to investigate the potential role for NAS and GABA in the pathophysiology of PPD.

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121. The Functional Neural Circuitry of Anhedonia in Adolescent Depression

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Background: Adolescent major depression (MDD) is a major public health concern, yet its pathophysiology remains poorly understood. The major challenge has been that the *categorical diagnosis of MDD*: *a)* relies on a syndromal definition that comprises a cluster of symptoms most likely derived from different etiologies, and *b)* does not capture the continuum of symptoms ranging from lesser to greater severity. Therefore, investigating symptoms may yield better results. Anhedonia – the reduced capacity to experience pleasure – is an ideal candidate for targeted biological investigation. While a core symptom of MDD, anhedonia is highly variable, manifesting a full range of severity in adolescent MDD. Anhedonia can be quantified and has been tied to the neural reward circuitry. To date, imaging studies in pediatric MDD populations have been relatively scarce; those reported have primarily made use of reward task-based fMRI-approaches, which is only able to reveal activation peaks. Further, none of these studies have taken into account the variability of anhedonia severity, resulting in only a partial view of the neural circuitry underlying anhedonia and reward. In the present study we extend prior work while using a resting-state functional connectivity (RSFC) approach, which is based on the brain's intrinsic activity during rest and permits identification of entire functional networks. Our aims were to examine striatum-based neural circuitry in adolescents with MDD compared to healthy controls (HC) and to relate anhedonia severity to specific circuits. We hypothesized that distinct striatum-based circuits would be related to anhedonia severity and to the categorical diagnosis of MDD.

Methods: Subjects:Twenty-one adolescents with adolescent-onset MDD and 21 HC (ages 13-19) were enrolled. A clinician met with parents and evaluated adolescents using a standard semi-structured interview, CDRS-R, and self-administered BDI. Anhedonia scores were derived from specific questions on the CDRS-R and BDI. MDD subjects met full DSM-IV-TR criteria, had episode durations 8 weeks, CDRS-R scores 39, and were psychotropic medication-free 3 months prior to the scan. HC did not meet criteria for any DSM-IV-TR diagnosis and were psychotropic medication-naïve. Data Acquisition: A 3T scanner was used to acquire 197 contiguous echo planar imaging functional volumes (TR = 2s, 39 slices) during rest with eyes open. A high-resolution T_1-weighted 3D anatomical image was also acquired using a magnetization prepared gradient echo sequence for spatial normalization and localization. ROI: We used a probability-weighted approach with seeds in the caudate, putamen and nucleus accumbens (NAc). To fully map striatum-based circuitry we also utilized a previously validated seed set of six striatal sub-regions. Preprocessing: Using FMRIB software library (FSL), slice time correction for interleaved acquisitions, motion correction, spatial smoothing, temporal bandpass filtering, and spatial normalization were performed. Nuisance signals were then removed and the residual mean time series for each seed was extracted. RSFC Analyses:For each seed region, the time series was extracted and voxel-wise maps were generated, indicating correlation strength. MDD adolescents and HC were compared via FSL using a random-effects model covarying for age, handedness, and sex; Gaussian Random Field Theory was used to correct for multiple comparisons at the cluster level ($Z > 2.3$; $p < 0.05$ corrected). These analyses yielded within- and between-group thresholded Z score maps of the positive and negative RSFC for each seed.

Results: Consistent with our hypothesis, group analyses revealed that MDD adolescents had increased RSFC between bilateral

striatal seeds (NAc, caudate and putamen) and the dorsomedial prefrontal cortex (dmPFC) and anterior cingulate cortex (ACC) compared to HC. Significant positive relationships were observed between anhedonia scores and bilateral caudate RSFC with the supplementary motor area, as well as right caudate and putamen RSFC with the perigenual ACC. Significant negative relationships were observed between anhedonia scores and left NAc RSFC with the the subgenual ACC and left caudate.
Discussion: Our findings are consistent with past observations of dmPFC and ACC alterations in MDD. As hypothesized, distinct alterations in circuits linking the striatum and reward system were observed in our dimensional analysis of anhedonia which were not detected in the categorical MDD analysis. Our results underscore the complementary role of these two approaches and the need to take symptom severity into account in biological research of psychiatric disorders.
Disclosure: V. Gabbay: None. **B. Ely:** None. **S. Bangaru:** None. **F. Castellanos:** None. **M. Milham:** None.

122. Structural Plasticity of the Right Inferior Frontal Gyrus in the Course of Bipolar Disorders - Interplay between Compensatory Changes, Illness Burden and Lithium Treatment
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Background: Bipolar disorders (BD) have a strong genetic underpinning and a typical onset in adolescence/early adulthood. Studies of unaffected as well as affected offspring of bipolar parents transitioning through the at-risk age (genetic high-risk design) are thus useful for separating biological vulnerability markers for BD from the effects of illness burden/medication exposure.
Methods: We performed a replication-design neuroimaging study. High-risk (HR) participants (aged 15-30 years) were recruited from families affected with BD in Halifax for the initial and in Prague for the replication study. They included 50 unaffected (30 in Halifax, 20 in Prague), 36 affected (21 in Halifax, 15 in Prague) subjects at genetic risk of BD, matched on age and sex with 49 controls (31 in Halifax, 18 in Prague) without personal or family history of psychiatric disorders. We also recruited bipolar subjects selected for substantial burden of illness (>10 years of illness, >5 episodes) and either at least 2 years of regularly monitored lithium exposure (Li group, $N=17$) or <3 months lifetime lithium exposure over 2 years ago (non-Li group, $N=12$). These BD patients were matched to 11 healthy controls. Structural imaging data from 1.5T magnets were analyzed using optimized voxel based morphometry in SPM8. Using a replication design, we first performed exploratory contrasts in the Halifax HR group to generate hypotheses, followed by replication tests in the Prague HR cohort as well as in the subjects selected for substantial burden of illness.
Results: Among the clusters of differences between the groups in the Halifax sample, increases in the right inferior frontal gyrus (rIFG), Brodmann area 47, which were present in both the affected and unaffected HR subjects relative to controls were replicated in both the affected and unaffected HR subjects relative to controls in the Prague cohort (replication corrected $p<0.001$). The clusters of increased rIFG relative to controls directly overlapped between the unaffected and affected subjects in each center. The rIFG volume negatively correlated with duration of illness. Furthermore rIFG was significantly smaller among subjects selected for substantial burden of illness and limited exposure to Li (non_Li group), but not among those with comparable illness burden and substantial exposure to Li treatment (Li group).
Discussion: The replicated finding of increased right inferior frontal gyrus gray matter volume in both affected and unaffected relatives of bipolar probands combined with a decrease in the same region in subjects selected for substantial illness burden, may indicate a compensatory upregulation of neurotrophic mechanisms, which is gradually overcome by the cumulative illness burden. The latter effect may be prevented by lithium treatment. The observation, that structural brain changes indicating neurobiological vulnerability for BD could be a result of an interplay between two opposing processes, has implications for early diagnosis, prevention and interpretation of neuroimaging findings in BD.
Disclosure: T. Hajek: Part 1: N/A, Part 2: N/A, Part 3: N/A, Part 4: N/A, Part 5: No. **J. Cullis:** None. **R. Blagdon:** None. **A. Duffy:** None. **L. Propper:** None. **M. Kopecek:** None. **T. Novak:** None. **C. Hoschl:** Part 1: Servier- research coordinator, Part 2: No, Part 3: No, Part 4: Eli Lilly & comp. – travel grant, Part 5: No. **M. Alda:** None.

123. Increased Glutamate in the Dorsal Anterior Cingulate Cortex is Associated with IFN-alpha-Induced Depression using Single Voxel Magnetic Resonance Spectroscopy
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Background: Despite strong evidence that activation of peripheral inflammatory responses may play a role in behavioral changes including depression, limited data is available on the brain metabolic mechanisms that underlie these behavioral alterations. Previous work in our laboratory has established that administration of the inflammatory cytokine, interferon (IFN)–alpha, to patients with hepatitis C virus (HCV) infection reliably causes symptoms of depression and fatigue. These behavioral changes have been associated with alterations in glucose metabolism in the basal ganglia as determined by positron emission tomography and altered task-induced neural activation in the dorsal anterior cingulate cortex using functional magnetic resonance imaging. Nevertheless, the relationship between cytokine-induced behavioral changes and changes in the metabolism of specific central nervous system cell types including glia and neurons in the basal ganglia and anterior cingulate cortex has yet to be determined. Therefore, we used single voxel proton magnetic resonance spectroscopy (MRS) to test the hypothesis that chronic peripheral administration of IFN-alpha leads to alterations in glial and neuronal function as well as excitatory neurotransmission, which in turn will be associated with behavioral changes such as depression and fatigue.
Methods: Eleven patients with HCV infection with no evidence of depression (as determined by SCID) and free of psychotropic medication or unstable medical conditions have participated in the study. Six patients underwent MRS scanning before and after four weeks of treatment with IFN-alpha. Five HCV patients who were awaiting IFN-alpha treatment served as controls and were studied in similar fashion, but no IFN-alpha was administered. Study assessments included the 17-item Hamilton Depression Rating Scale, the Multidimensional Fatigue Inventory, and the Epworth Sleepiness Scale. An anatomical T1-MPRAGE scan was obtained using Siemens Trim Trio Scanner to enable identification of voxels. The MRS settings were: TE/TE/NS = 3000/30/128, voxel sizes = 20x30x10 mm^3 in the dorsal anterior cingulate cortex and 17x30x17 mm^3 in the left and right basal ganglia. Post processing was done using the LC Model. Metabolite values including biomarkers of glial activation (myo-inositol, choline), excitatory neurotransmission (glutamate/glutamine) and neuronal viability (n-acetyl aspartate) were normalized using the metabolite/creatinine ratio.

Results: Compared to controls, the glutamate/creatinine ratio in the dorsal anterior cingulate cortex significantly increased following 4 weeks of administration of IFN-alpha ($p < 0.05$). This increase in glutamate/creatinine ratio was in turn significantly correlated with increases in depression severity ($r = 0.66$, $p < 0.05$). In addition, daytime sleepiness was positively correlated with the choline/creatinine ratio in the dorsal anterior cingulate cortex ($r = 0.85$, $p < 0.001$). No changes in brain metabolism were identified in the basal ganglia.
Discussion: The increased glutamate concentrations in the anterior cingulate cortex and its association with depressive symptom severity following administration of IFN-alpha suggests that IFN-alpha might lead to astrocyte dysfunction. Inflammatory cytokines have been shown to reduce glutamate transporters on astrocytes as well as increase astrocyte glutamate release. The increase in choline may be related to increased cell wall synthesis and turnover, possibly reflecting glial activation/proliferation, which in turn may be secondary to a central inflammatory response that we and others have shown can lead to sleep alterations.
Disclosure: E. Haroon: None. **R. Anand:** None. **X. Chen:** None. **S. Parekh:** None. **B. Woolwine:** None. **J. Spivey:** None. **X. Hu:** None. **A. Miller:** Part 1: Andrew H. Miller has served as a consultant for Abbott Laboratories, AstraZeneca, GlaxoSmithKline, Lundbeck Research USA, F. Hoffmann-La Roche Ltd., Schering-Plough Research Institute and Wyeth/Pfizer Inc., Part 2: None, Part 3: Andrew H. Miller has served as a consultant for Abbott Laboratories, AstraZeneca, GlaxoSmithKline, Lundbeck Research USA, F. Hoffmann-La Roche Ltd., Schering-Plough Research Institute and Wyeth/Pfizer Inc., Part 4: Andrew H. Miller has received research support from Centocor Inc., GlaxoSmithKline, and Schering-Plough Research Institute, Part 5: No.

124. Social Rejection activates Endogenous Opioid Systems

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Background: Endogenous opioids, which alleviate physical pain, are also thought to have broader effects on the regulation of responses to stressors, including social distress in several species including chicks, rats, guinea pigs, dogs, and monkeys. It is not known if endogenous opioids perform a similar function in humans. In this study, it was hypothesized that the endogenous opioid system would respond to social rejection, an explicit declaration that one is not liked, by measuring μ-opioid receptor binding *in vivo* at baseline and during social rejection. Reductions in receptor availability (i.e., binding potential, BP) are indicative of endogenous opioid release and μ-opioid receptor activation.
Methods: 10 healthy volunteers (7 females; mean age, 33 ± 11 years) were clear of active medical illness, current or past psychiatric disorders, and had no history of medication at the time of study. [^{11}C]carfentanil, a selective and specific μ-opioid receptor radioligand, was intravenously administered during positron emission tomography (PET). BP was calculated using Logan plots with occipital cortex as a reference region. Participants completed online ratings for profiles of the opposite sex, and only the most-liked profiles were presented to increase feedback saliency. During the scan, participants viewed these profiles along with feedback that they were not liked (12 profiles/block). Baseline blocks contained a similar visual presentation with no feedback. Blocks were presented in a randomized, counterbalanced design across participants, and measures of emotional state were obtained after each feedback. *A priori* regions of interest included the ventral striatum, amygdala, thalamus, insula, anterior cingulate cortex, and periaqueductal gray (PAG). These regions have high levels of μ-opioid receptors and have been previously shown to respond to physical pain.
Results: Measures of emotional state indicated that participants felt significantly more "rejected" during rejection compared to baseline blocks ($P = 0.006$). Reductions in μ-opioid binding potential was found during rejection compared to baseline blocks in the bilateral ventral striatum (left, $P = 0.002$; right, $P = 0.003$), left amygdala ($P = 0.006$), midline thalamus ($P = 0.006$), bilateral insula (left, $P = 0.02$; right, $P = 0.01$), right anterior cingulate cortex ($P = 0.03$), and PAG ($P = 0.03$). In contrast, no significant decreases in binding were found during baseline compared to rejection blocks.
Discussion: This is the first demonstration that the endogenous opioid system responds to social rejection. The PAG, midline thalamus, amygdala, ventral striatum, insula, and anterior cingulate comprise a pathway by which stress-related information may influence emotion, mood, and motivation. Decreased μ-opioid BP reflects the release of endogenous opioids interacting with μ-opioid receptors, which may function to regulate activity along this pathway. Negative repercussions of social rejection include lowered self-esteem, major depressive disorder, social anxiety disorder, substance abuse disorders, risk-taking behavior, and aggression. The results suggest that endogenous opioids are involved in the link between social rejection and the potential to develop these psychiatric illnesses and maladaptive behaviors.
Disclosure: D. Hsu: None. **T. Love:** None. **H. Wang:** None. **K. Meyers:** None. **K. Hazlett:** None. **L. Ni:** None. **S. Walker:** None. **S. Korycinski:** None. **R. Koeppe:** None. **J. Crocker:** None. **S. Langenecker:** None. **J. Zubieta:** Part 1: Eli Lilly, Abbott, Merk, Johnson & Johnson, Part 2: Eli Lilly, Part 3: Eli Lilly, Part 4: None.

125. Brain Gray and White Matter Abnormalities identified with Ex Vivo Magnetic Resonance Imaging in the GT-tg Mouse after HIV1-Tat Expression

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Background: Recent studies suggest that HIV-dementia may be due in part to neurotoxic actions of HIV-accessory proteins such as Tat. HIV has been associated with cognition and mood disorders. We hypothesized that Tat protein expression is sufficient to mediate neurodegeneration in brain areas involved in those disorders. Using the bigenic GT-tg mouse, we induced brain selective Tat expression with doxycycline (Dox) and measured brain abnormalities using ultra high magnetic field (9.4 Tesla) ex vivo structural magnetic resonance imaging, combined with voxel based morphometry (VBM) and diffusion tensor imaging (DTI) analyses.
Methods: Subjects were adult male GT-tg bigenic (Kim *et al.*, Am J Pathology 162:1693, 2003) and C57Bl/6J wild-type (Jackson Labs) mice. GT-tg bigenic mice came from a colony established from 2 breeding pairs homozygous for the Tat allele (a gift of Dr. JJ He). Dox treatment induces expression of the Tat86 gene via a tetracycline transactivator located exclusively in GT-tg mouse brain astrocytes. GT-tg bigenic mice were administered Dox via intraperitoneal injection once/day (100 mg/kg in 0.9% saline, 0.3 ml/30 mg body weight) for 5 or 7 days. After treatments, mice ($n = 8$/group) were perfused with 4% paraformaldehyde and ProHance (Gadoteridol, Bracco Diagnostics, Inc., volume ratio of 20:1, Cyr *et al.*, Neuroimage 26:83,2005). Ex vivo imaging was conducted on a Varian 9.4 Tesla scanner using a proton surface coil. Imaging was performed with these scan parameters: Repetition Time (TR) = 3033ms, Echo Train Length (ETL) = 8, matrix size = 512x512, Field of View (FOV) = 20x20mm, Echo Time (TE) = 25.9ms, averages = 64, slice number = 28, slice thick-

ness = 0.5mm, ogap, in-plane resolution = 39x39um, scan time-3.33h. Diffusion imaging was performed using a fast-spin echo-multi-slice DTI protocol (Boretius *et al*., Cereb Cortex 19: 2838, 2009; Lehmann *et al*., Exp Neurol. 223: 238, 2010) as follows: TR = 4000 ms, ETL = 8, matrix size = 256x256, FOV = 20x20mm, Effective TE = 27 ms, averages = 8, diffusion gradients were applied at 6 noncollinear directions, diffusion gradient amp = 18G/cm, duration = 5 ms, separation = 11.5 ms, b-value = 1140 mm^2/s, scan time = 1.75h. VBM image analyses were performed in FSL v.4.1 (FMRIB Software Library). Brains of uninduced GT-tg and Dox-treated C57Bl/6J control mice were co-registered to a selected control brain and then averaged to create a study-specific template (Sawiak *et al*., Neurobiol Dis. 33: 20, 2009) to which brains from induced GT-tg mice were compared. Signal intensities from aligned images were normalized to the mean image pixel value to minimize scan-to-scan variance. Registered images were spatially smoothed using a 0.05 mm Gaussian kernel. The permutation-based nonparametric inference processing algorithm was executed in FSL via the "randomize" command, creating nonparametric structural difference distribution maps. DTI calculation was performed using TrackVis DTI software (http://trackvis.org, Martinos Center, Mass. General Hospital). The linear least square fitting method was used. Fractional Anisotropy (FA) maps were generated. VBM and FA maps were compared using MRIcro analysis software (Rorden & Brett, Behav Neurol. 12: 191, 2000), and significant differences were identified with cluster corrected t-tests with a threshold set at $p \leqslant 0.05$.

Results: Significant gray matter density reductions were found in the amygdala, amygdala-hippocampal area, piriform, perirhinal, and entorhinal cortices in 5-day Tat-induced mice compared to Dox-treated C57Bl/6J mice (corrected $p \leqslant 0.05$, two-tailed t-test). Significant FA decreases were detected in the insula, endopiriform nucleus, and part of the striatum in 7-day Tat-induced compared to uninduced GT-tg mice (corrected $p \leqslant 0.05$, two-tailed t-test).

Discussion: These initial cross sectional ex vivo neuroimaging findings suggest that induction of brain-specific Tat expression in the GT-tg bigenic mouse is neurotoxic and is sufficient to reduce gray matter density and FA in brain areas involved in learning and memory and in mood disorders. The data support the concept that therapeutics targeting brain Tat protein may have utility for moderating some of the brain structural abnormalities and possibly some of the behavioral abnormalities and cognitive deficits observed in HIV-infected individuals. Our findings warrant additional studies to further characterize Tat's effects including longitudinal behavioral and imaging studies.

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Disclosure: A. Carey: None. **X. Liu:** Part 1: General Electric, Part 2: General Electric, Part 3: General Electric, Part 4: General Electric, Part 5: General Electric. **E. Sypek:** None. **J. McLaughlin:** Parts 1-5: Torrey Pines Institute for Molecular Studies. **M. Kaufman:** Part 1: Varian (Agilent), Air Products and Chemicals, Inc., Michael J. Fox Foundation for Parkinson's Research, Torrey Pines Institute for Molecular Studies, NARSAD, GSK, Part 4: Varian (Agilent), Air Products and Chemicals, Inc., Michael J. Fox Foundation for Parkinson's Research, Torrey Pines Institute for Molecular Studies, NARSAD, GSK.

126. CANDIShare: Enabling Probabilistic Neuroanatomy in Child Psychiatry

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Background: There are numerous psychiatric disorders that can plague the development of children. Each of these disorders manifests as a distinct pattern of clinical, behavioral, etiological, neuroanatomic and neurofunctional characteristics that challenge the management of the individual patient, as well as the development of successful intervention and prevention strategies. In the area of neuroimaging, a substantial number of studies have been performed to date; and while much has been learned from this investment, this represents only the tip-of-the-iceberg of the information that can be gleaned from the data. The Child and Adolescent NeuroDevelopment Initiative (CANDI) at University of Massachusetts Medical School is making available a series of structural brain images, as well as their anatomic segmentations and demographic data, in a coordinated set of related morphometric resources.

Methods: The initial data set is a release of 103 subjects (T1-weighted MRI scans and anatomic segmentation) that comprised the neuroanatomic data published in 2008 in an article by Frazier, *et al*. Schizophr Bull. 2008 Jan;34(1):37-46. The subjects include 57 males and 46 females, aged 4-17, and come from four diagnostic groups: Healthy Controls (N = 29), Schizophrenia Spectrum (N = 20), Bipolar Disorder with Psychosis (N = 19), and Bipolar Disorder without Psychosis (N = 35). Images were acquired from 1998 – 2005 at the McLean Hospital Brain Imaging Center on a 1.5 Tesla General Electric Signa Scanner. Structural imaging was performed using a three-dimensional inversion recovery-prepared spoiled gradient recalled echo in the coronal plane with 124 1.5 mm thick slices, repetition time 10 ms, TE 3, flip angle 25°, field of view 24 cm, acquisition matrix 256×192 and 2 excitations. Each subject from each of the diagnostic groups have under non-linear registration (FSL: FNIRT) to the MNI152 space, and probabilistic images and segmentation results have been generated and are available.

Results: The complete data release is hosted at: http://www.nitrc.org/projects/candi_share/. The MR images released have undergone analysis at the Center for Morphometric Analysis (CMA) at the Massachusetts General Hospital which includes: preprocessing ('positional normalization' to put the image into the standard orientation of the Talairach coordinate space, and bias field correction) and 'general segmentation' following the CMA segmentation protocol. Segmented regions include: Cerebral Cortex and White Matter, Cerebellum Cortex and White Matter, Lateral Ventricle, Thalamus, Ventral Diencephalon, Caudate, Putamen, Pallidum, Accumbens, Hippocampus and Amygdala, bilaterally; as well as Brain Stem, 3rd and 4th ventricles. Basic demographic details for each subject include diagnosis, age, gender, and handedness. Groupwise probability data are procided as a seperate 'release'. The release is provided under the Creative Commons: Attribute license, and is structured as four bundles (tar) of imaging data, one for each diagnostic group. Within each bundle, there are separate directories for each subject that includes the data. Release version V1.0 includes only the imaging data and basic demographics and is accessible with no limitations. Version V1.1 adds the segmentation data as an indexed label file in register with the MR image for each subject, and requires provision of a contact email address (via the NITRC registration process) and requires a 'click-through' acceptance of the licensing terms.

Discussion: This image and segmentation data release for a specific publication represents the first in a series of data representing the pediatric brain in health and disease from data collected in our lab over the past ten 15 years. Gropuwise probability data is valuable for generation of *a priori* statistics for automated segmentation procedures, and the individual data is suitable for pooling with other data collections. This release of information is designed to be dramatically greater than merely 'making the images available': each image is associated with substantial analytic results, many of which have been utilized in the preparation of various publications and comparisons. Moreover, these data will be most effectively shared with the research community when shared in a way that preserves the linkages between the images, the resultant analytic data and meta-data, and

its relationships to other public sources of related information. In short, this represents a 'Knowledge Management' environment that will facilitate traversal of these data and linkages.
Disclosure: D. Kennedy: Part 1: TCG, Inc. Washington, DC., Part 2: TCG, Inc. Washington, DC., Part 3: TCG, Inc. Washington, DC. **S. Hodge:** None. **C. Haselgrove:** Part 1: Neuromorphometrics, Inc. Somerville, MA, Part 2: Neuromorphometrics, Inc. Somerville, MA, Part 3: Neuromorphometrics, Inc. Somerville, MA, Part 4: none, Part 5: no. **P. Rane:** None. **J. Frazier:** Part 1: Bristol-Myers Squibb, Glaxo Smith Kline, Neuropharm, Otsuka America Pharmaceutical, Pfizer, Inc. and Seaside Therapeutics., Part 4: Bristol-Myers Squibb, Glaxo Smith Kline, Neuropharm, Otsuka America Pharmaceutical, Pfizer, Inc. and Seaside Therapeutics.

127. Can ASL Measures of Anterior Cingulate Cortex Perfusion Predict Treatment Outcome in Major Depressive Disorder?

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Background: Major Depressive Disorder (MDD) is a common condition that is a major public health threat. There are numerous treatments for MDD, but finding the treatment that will successfully treat a patient often requires multiple trials. Even after multiple trials, however, many patients never achieve remission. Identification of biological brain markers, or "phronotypes," that are associated with treatment outcome in MDD holds the promise of better targeting of treatments and directing research to develop novel intervention approaches. In prior work, we demonstrated that connectivity as measured by correlation in non-task fMRI BOLD signal between the left rostral anterior cingulate and left subcallosal cingulate was associated with treatment outcome in participants with MDD who were treated with an antidepressant for 8 weeks. Using arterial spin labeling (ASL) to measure brain perfusion, we tested whether perfusion measures in the left rostral anterior cingulate (LACC) and left subcallosal cingulate (LSC) were associated with treatment outcome in this same cohort. We hypothesized that since the connectivity measures between these structures were negatively correlated, the difference in perfusion measures would correlate with treatment outcome as well.
Methods: Participants with non-psychotic MDD and no significant co-morbidities were recruited from the community and other research protocols. After informed consent was obtained, participants underwent screening and clinical evaluation. A baseline ASL MRI was subsequently obtained. Participants were free of all medications when scanned. Images were acquired using a research-dedicated 3T Philips Achieva scanner (Philips Medical System, Netherlands)with an eight-channel SENSE head coil. Participants were instructed to hold still, keep their eyes open, and focus on a cross in the middle of the screen. The ASL MRI scan used the pseudo-continuous labelling technique with a labelling duration of 1650ms and post-labelling delay of 1525ms. Background suppression was performed in combination with the ASL imaging. Other imaging parameters were: TR/TE = 4000ms/14ms, in-plane resolution 3x3mm^2, 17 slices with 7mm thickness which cover the whole brain, 60 dynamics, with scan duration of 4 minutes. After scanning was completed, participants were treated with an antidepressant outside of this study. After 8 weeks of treatment, they were clinically re-evaluated. The primary treatment outcome was defined as the percent change in QIDS-SR from baseline to week 8 with a response being defined as a 50% improvement. The ASL data was motion-corrected using SPM2. Then an in-house MATLAB program was used to create perfusion maps for each individual brain. Using FSL, the mean grey matter perfusion value for the LACC and LSC were measured for each subject. The mean perfusion measure for the LSC was subtracted from the mean perfusion measure for the LACC [DIFF = LACC – LSC]. To test if this measure (DIFF) was associated with treatment outcome, a regression was performed using SPSS with DIFF being the predictor and percent change QIDS-SR being the dependent variable. The relationship between perfusion measures and clinical outcome was also graphed.
Results: Thirteen (10 female, mean age 33.7, s.d. 7.4, range 22-48 years) of the seventeen participants enrolled had adequate imaging and clinical data. Ten participants took buproprion SR 150 mg twice a day as part of a clinical trial, two took escitalopram 20 mg once a day, and one took aripiprazole 5 mg once a day. Seven of the 13 who were evaluated at eight weeks met criteria for response.
A regression with the predictor being difference in perfusion for LACC minus LSC and the dependent variable being percent change in QIRS-SR score produced an t-value of 1.585 ($p = 0.141$) with an R^2 of 0.186. Looking at the individual regions as predictors using a linear regression, the LACC was $t = 01.660$, $p = 0.128$, while the LSC was $t = -0.827$, $p = 0.428$.
Discussion: In this preliminary work, the association between the difference in perfusion from the LACC and LSC to the treatment outcome was found to not quite reach significance defined as a two-tailed $p < 0.05$. Despite this, 19% of the variance in treatment outcome was accounted for by this technique making this an interesting possibility for evaluation in a larger study. The perfusion of the LACC appears to contribute most of the treatment prediction in this sample. Further work will be required to more adequately test this hypothesis. Measures of perfusion using ASL offers another modality that can be easily acquired during an MRI. Successful treatment prediction will likely require multiple modality measures to provide clinically useful information.
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128. Sertraline-Induced Normalization of Medial Visceromotor Network Dysfunction in Major Depression

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Background: Depression is associated with elevated mortality rates in several disease contexts yet the mechanisms are not well

understood. Depression is associated with reduced vagal tone as measured by heart rate variability (HRV) as well as dysfunction of the medial prefrontal cortex, a key node in the neural network regulating autonomic and other vegetative functions. To evaluate the covariation of these abnormalities, we examined the relationship over time between functional magnetic resonance imaging (fMRI) Blood Oxygen Level Dependent (BOLD) activity, vagal tone and depressive symptoms in depressed patients treated with sertraline and healthy volunteers.
Methods: Ten patients with major depression (MDD) with a Hamilton Depression Rating Scale 17 score of 16 or greater and 10 untreated control subjects were enrolled in a 12-week protocol. Subjects (9 females in each group) were right-handed, medically and neurologically healthy, not taking centrally-acting medications and 18-60 years of age (controls matched to patients within 5 years). After week 0 assessment patients were treated with escalating doses of sertraline up to 200 mg based on Montgomery-Asperg Depression Rating Scale (MADRS) scores, which were assessed weekly over 12 weeks. All subjects had structural and fMRI at weeks 0, 2, 6 and 12. fMRI imaging on each occasion involved three affective processing tasks over 31 minutes. Functional images were acquired on a GE 3T scanner (TR = 3.0 seconds) using a spiral in-out protocol acquired in coronal orientation to minimize signal loss in the ventral frontal lobe due to magnetic susceptibility artifact. The three tasks targeted emotional responses that were implicit, in the attentional background or in the attentional foreground, respectively, and included 1) implicit vs. explicit perception of fearful faces, 2) the Emotional Counting Stroop (four conditions including pleasant, unpleasant, depression-specific, and neutral), and 3) an Attention to Emotion task that involved viewing pictures from the International Affective Picture System and rating pleasantness in one condition and spatial location (indoor-outdoor) in the other. Continuous ECG (later pruned for artifact) was obtained in synchrony with fMRI. A "moving window" analysis was performed in which high frequency HRV (a relatively pure measure of vagal tone) was assessed over a 16-second period (or window), recalculated every 3 seconds thereafter and then paired with each new BOLD image acquired every 3 seconds across the 31-minute fMRI protocol (about 620 paired values of BOLD and HRV in each imaging session). This analysis capitalized on variability in brain and autonomic function across tasks and did not examine task-specific correlations.
Results: All control subjects scored 4 or less on the MADRS throughout the study whereas patients showed significant declines (MADRS mean = 21.6 at week 0, MADRS mean = 9.3 at week 12, $p < .003$). Control subjects showed positive BOLD-HRV correlations in medial prefrontal cortex in each of the 4 imaging sessions over 12 weeks ($p < .005$). By contrast, depressed patients showed negative BOLD-HRV correlations in medial prefrontal cortex at week 0 ($p < .005$ uncorrected) and positive correlations in medial prefrontal cortex at weeks 6 and 12 ($p < .005$). By week 12, relative to week 0, depressed patients also showed significant positive BOLD-HRV correlations in bilateral anterior insula, dorsal anterior cingulate cortex and periaqueductal gray ($p < .005$ uncorrected).
Discussion: The results in the control group replicated the well-established normative pattern of a positive correlation between medial prefrontal cortex activity and HRV. In depressed patients at week 0 the negative correlation between HRV and BOLD activity in prefrontal areas, both independent of and relative to controls, suggests that in depression prefrontal hyperactivity is associated with vagal tone deficits and a failure to properly regulate vagal tone. The reversal of BOLD-HRV correlations to a normative pattern by week 12 suggests a restoration in the function of the medial visceromotor network with antidepressant treatment. These findings provide a new lead in understanding and potentially ameliorating a key mechanism by which MDD contributes to adverse health outcomes.
Disclosure: R. Lane: None. **T. Wager:** None. **R. Mercado:** None. **S. Schafer:** None. **J. Thayer:** None. **J. Allen:** None.

129. Amygdala and Subgenual Cingulate Activation to Emotion Processing is Related to Cortisol Reactivity to Stress

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Background: There is a growing literature suggesting that reactivity to emotional stimuli is highly variable across individuals and may be a risk factor for psychiatric illness. Less well studied are relationships between cortisol, a stress hormone, and individual differences in activation during emotional probes with fMRI. The present study investigated the premise that areas known to be abnormally elevated during emotion processing for those with depression and anxiety may likewise be elevated in healthy individuals who show greater reactivity to stress challenge. We hypothesized that individuals with evidence of increased stress reactivity to challenge, via mean salivary cortisol and decline in cortisol over the course of an fMRI experiment, would exhibit increased activation in amygdala, insula, and subgenual and rostral anterior cingulate cortices, with reduced activation in hippocampus and lateral prefrontal cortices.
Methods: Twenty-five individuals (mean age 34), including sixteen women, were screened using a neurological and psychiatric screen and the SCID-IV and were free of any Axis I or II disorder for self or for first degree relatives. They completed an explicit facial emotion identification task, with a control task requiring categorization of animals. Analyses of interest for the present investigation were a block design analysis of faces minus animals, and of fearful faces minus neutral faces. Average cortisol across the pre and post session, and percent decline from pre- to post-session cortisol measures were used as regressors in separate linear regression analyses. Analyses were conducted using whole brain corrected threshold of $p < .05$, based upon height and extent with AlphaSim.
Results: Activation for the main contrasts indicated bilateral ventro-lateral frontal and middle temporal areas for the Faces-Animals contrast. A parietal, posterior temporal, superior frontal, and insular network was active for the Fear-Neutral contrast. Select results include higher average cortisol levels associated with increased activation for the Faces-Animals contrast in the rostral anterior cingulate and medial prefrontal cortex, whereas lower cortisol was correlated with activation in posterior hippocampus and fusiform gyrus. Those with highest pre-scan cortisol and greatest decline from pre-post- scanning measures exhibited increased activation for Faces-Animals in subgenual anterior cingulate and posterior hippocampus and fusiform bilaterally and for Fear-Neutral in right amygdala, uncus, ventral striatum, and orbital frontal cortex, as well as ventral tegmental area.
Discussion: The present results suggest that fMRI is a stress challenge for some individuals, and that those individuals tend to show increased activation in areas known to be abnormally activated in those with mood and related disorders, including subgenual and rostral anterior cingulate, amygdala, and medial prefrontal cortex. Furthermore, those with lowest reactivity are able to engage classic face processing areas to a greater extent, including fusiform gyrus and hippocampus. Studies of this type may increase understanding of acute cortisol reactivity to stress as a risk marker for illness.

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130. White Matter Structural Abnormalities in Bipolar Illness Revealed Using Diffusion Weighted MR Imaging

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Background: Bipolar illness is characterized by extreme mood swings, including both manic and depressive episodes commonly accompanied by psychosis. Many imaging studies have investigated white matter changes in bipolar illness, and the results have suggested abnormal intra- and inter-hemispheric white matter structures, particularly in the fronto-limbic and callosal systems. However, some inconsistency remains in the literature, and no study to-date has utilized brain network analysis using graph theory. In graph theory, a network is a set of nodes and the connections between them. The advantage of such approach is that it allows for quantitative analyses of complex brain networks to provide information on organizational systems. We hypothesized that true positive white matter changes should exhibit alterations in both DTI and high angular resolution diffusion imaging (HARDI) based measures, which can be confirmed with brain network analyses.
Methods: *Subjects:*Participants included subjects with bipolar disorder (n = 24; 14 male and 10 female; age: 42.9 +/- 13.1) and healthy controls (n = 20; 10 male and 10 female; age: 43.3 + /- 11.1). All bipolar subjects met the DSM IV criteria for bipolar I disorder, and were euthymic at the time of the study. The two groups did not statistically differ in either gender or age. Diffusion weighted MRI data were acquired on a Siemens 3T Trio scanner. Sixty contiguous axial brain sliceswere collected using the following parameters: 64 diffusion-weighted ($b = 1000 s/mm^2$) and 1 non-diffusion weighted scan; FOV: 190mm by 190mm; voxel size: 2x2x2mm; TR = 8400ms; TE = 93ms). For each subject, we parcellated the whole brain white matter into 50 ROIs by registering the corresponding bo image to the ICBM DTI-81 white matter atlas (http://www.loni.ucla.edu/Atlases/Atlas_Detail.jsp?atlas_id = 15) using 12p affine transformations. White matter integrity measures based on both DTI and HARDI were computed and averaged in each ROI for group comparisons. In addition, we computed whole-brain probabilistic tractography using in-house programs based on HARDI and conducted cortical gray matter parcellation to yield 66 ROIs using freesurfer. The gray matter parcellation was performed in the MPRAGE space, and the resulting ROI labels were registered to each subject's DTI space using affine transformations in FSL. For each pair of ROIs, the number of fibers connecting them determined the element in the corresponding connectivity matrix. These matrices were analyzed using the Brain Connectivity Toolbox to yield several graph theory metrics (network strength and the normalized clustering coefficient, path length and global efficiency).For all measures, we performed ANCOVAs to compare between the two groups by controlling for gender and age.
Results: For white matter ROI analyses, the only group differences (as evidenced in both DTI and HARDI metrics) were in the right tapetum of corpus callosum (CC). It exhibited lower FA ($p = 0.006$), lower linear and planar anisotropy ($p = 0.007$ and 0.026), as well as elevated MD ($p = 0.021$) and HARDI-corrected MD ($p = 0.036$). These results overall suggest an impaired corpus callosal system, especially in the right hemisphere. For brain network analyses, there were no group differences in global graph-theoretic metrics. To further investigate the role of CC in the context of network analyses, we devised two novel metrics (m1 and m2) that measure the inter- and intra-hemispheric integration. Here m1 (m2) is the mean number of ipsilateral (contralateral) nodes travelled by the shortest path connecting any two nodes in the same hemisphere. To compute shortest paths, the distance matrix was defined as the element-wise inverse of the square root of the connectivity matrix. Our results supported a laterality effect of m1 in normal controls (0.0145; left vs right), which was attenuated in bipolar subjects (-0.007) with statistical significance ($p = 0.048$).
Discussion: This study represents the first brain network analysis in bipolar illness. ROI white matter analyses confirmed white matter abnormalities in the corpus callosum in the form of impaired integrity (especially in the tapetum). Moreover, brain network analyses suggested altered laterality in intra-hemispheric integration. In particular, the results supported the presence of natural laterality in intra-hemispheric integration in normal controls, which is attenuated in bipolar illness possibly explained by a less efficient network integration within the right hemisphere.
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131. Monoamine Oxidase A (MAO-A) Binding in Prefrontal and Anterior Cingulate Cortex in Postpartum Depression

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Background: With a prevalence rate of 13 percent, postpartum depression (PPD) represents the most common complication of childbearing. Levels of monoamine oxidase A (MAO-A), have shown to be elevated in major depressive disorder, but this has not been evaluated in PPD. We hypothesize that MAO-A is elevated in PPD, but through a different mechanism. We previously showed an increase in MAO-A binding in early postpartum in healthy women (Arch Gen Psych 2010) and hypothesize in the present study that MAO-A binding in prefrontal and anterior cingulate cortex will normalize in healthy women in the first 1.5 years postpartum while it will be elevated in women who suffer from PPD within the first 1.5 years postpartum.
Methods: Fifteen postpartum depressed women and 15 postpartum healthy women underwent [^{11}C] harmine positron emission tomography (PET) to measure MAO-A total distribution volume (MAO-A V_T), an index of MAO-A levels. All were medication free, and had no other psychiatric or medical illnesses.
Results: A multivariate analysis of variance showed a main effect for group (postpartum depressed versus healthy) upon prefrontal and anterior cingulate cortex MAO-A V_T ($F_{2,27} = 4.9$, $p = 0.026$). Individual t-tests revealed a significant elevation in MAO-A V_T in

prefrontal cortex (mean difference: 18%, $p < 0.004$) and anterior cingulate cortex (mean difference: 18%, $p < 0.009$) in postpartum depressed women compared to postpartum healthy women.
Discussion: We identify elevated MAO-A binding in prefrontal and anterior cingulate cortex as an important mechanism in the neurobiology of major depressive episodes with postpartum onset. Our findings argue for clinical trials of MAO-A inhibitors in PPD as well as developing preventative strategies against persistently elevated MAO-A levels in early postpartum.
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132. Genetic Variation of the 5-HT_{2C} Receptor Modulates Human Striatal Dopaminergic Activation to Stress

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Background: Serotonin 2C (5-HT_{2C}) receptors are expressed in the dopaminergic midbrain and striatum, where they mediate the inhibitory effects of serotonin on mesoaccumbal and nigrostriatal function in experimental animals. Accordingly, preclinical findings have implicated 5-HT_{2C} receptors in motor function, the behavioral and neural effects of drugs of abuse, and the actions of antidepressant and antipsychotic drugs. In humans, a single nucleotide variant (rs6318, Cys23Ser) of the 5-HT_{2C} receptor gene (*HTR2C*) has been associated with greater constituent activity and with mood disorders.
Methods: We examined the effects of this *HTR2C* variant on striatal dopaminergic function in 54 healthy humans using positron emission tomography and the displaceable D_2/D_3 receptor radiotracer [^{11}C]raclopride. Binding potential (BP_{ND}) was quantified before and after a stress challenge consisting of 20 minutes of moderate sustained pain, and changes in BP_{ND} were interpreted as dopamine release. The Cys23Ser variant was genotyped directly on a custom array, and ancestry informative markers were used to control for population stratification.
Results: Carriers of the Ser23 allele demonstrated greater stress-induced dopamine release in the nucleus accumbens ($p = 0.03$), caudate nucleus ($p = 0.007$), and putamen ($p = 0.07$ trend) after controlling for age, sex, and ancestry ($p = 0.03$, multivariate test with 3 regions). The Cys23Ser variant accounted for 6–12% of the variance in dopamine release. There was no effect on baseline BP_{ND} ($p > 0.05$).
Discussion: Our findings indicate that a genetic variant of *HTR2C* with putatively greater constituent activity (Ser23) causes greater stress-induced release of dopamine in the striatum in humans. The mesoaccumbal and nigrostriatal projections in Ser23 carriers may be subject to greater tonic serotonin-mediated inhibition, altering the dynamics of the system during stress. Stress-induced dopamine release may mediate the effect of *HTR2C* variation on risk of mood disorders.
Disclosure: B. Mickey: Part 1: No company or funding agency played a role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the abstract/poster. **B. Sanford:** None. **T. Love:** None. **P. Shen:** None. **C. Hodgkinson:** None. **D. Goldman:** None. **J. Zubieta:** Part 1: Dr. Zubieta has served as a paid consultant for Eli Lilly and Co., Johnson & Johnson, Merck, and Abbott in the 3-year period prior to submission. No company or funding agency played a role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the abstract/poster.

133. Lower Anterior Corpus Callosum Fractional Anisotropy in Major Depressive Disorder as Measured by Tract Based Spatial Statistics and Diffusion Tensor Imaging

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Background: Major Depressive Disorder (MDD) has been associated with structural and functional abnormalities throughout the prefrontal cortex and anterior cingulate among other regions. Several authors have suggested abnormalities in the white matter (WM) tracts that connect these regions to the rest of the brain in both early and late onset MDD. Several recent reports examined the role of functional connectivity using resting state fMRI. Alternatively, it is possible to measure connectivity using Diffusion Tensor Imaging (DTI). DTI is an MRI pulse sequence used to study WM *in-vivo* to evaluate fiber integrity and orientation. Several macro parameters describing the local organization of the tissue, such as the Fractional Anisotropy (FA) or the Mean Diffusivity (MD) can be computed. Tract Based Spatial Statistics (TBSS) is a part of the FSL library which allows voxel level cross-subject analysis by projecting each subject's FA onto a common skeleton computed on the average FA map. We hypothesized that WM integrity in MDD as measured using TBSS would be disrupted compared to healthy volunteers.
Methods: *Participants:* Forty-two subjects (age 30.5 ± 9.6) with SCID diagnosed DSM-IV MDD and 27 healthy volunteers (age 35.0 ± 11.3) were recruited. MDD subjects had to have a 17-item Hamilton Depression score (HAMD) of at least 15 to enter the study. The Institutional Review Board of the New York State Psychiatric Institute approved the protocol. Subjects gave written informed consent after an explanation of the study. *Image Acquisition:* MRI acquisition was performed on a GE Signa 3.0T scanner with the following settings: 25 non collinear directions, 5 non weighted images, b value 1000 s/mm^2, slice thickness 3 mm, matrix 256x256 (voxel size 0.9375x0.9375x3 mm^3), field of view (FOV) 240x240 mm^2, repetition time (TR) 14 s, echo time (TE) 84.4 ms. *TBSS analysis:* DTI preprocessing included skull stripping using the Brain Extraction Tool (BET) and Eddy current correction tool included in the FMRIB Software Library (FSL, Oxford, U.K.). Next, the diffusion tensor model was fit to each voxel using CAMINO and the FA map was obtained. TBSS analysis of the FA maps included several steps: 1) the individual FA maps were non-linearly aligned to a common FMRIB58 FA template; 2) aligned FA maps were averaged to obtain a study mean FA map; 3) the study skeleton, i.e. the map including only the center of each WM tract, was computed on the mean FA map; 4) the study skeleton was thresholded (FA0.4) to maintain only WM voxels; 5) each individual FA map was projected on the common FA skeleton to obtain the individual FA skeleton; 6) Voxel level analysis was performed on the individual FA skeletons to assess for the differences between the two populations. A threshold of $p < 0.05$ corrected for multiple comparison (False Discovery Rate) was applied to test both when the FA was decreased in patients versus controls and when the FA was increased.
Results: Group-wise comparisons using TBSS were performed for both cases ($FA_{Control} > FA_{Patient}$ and $FA_{Patient} > FA_{Control}$) separately. Significant difference ($p < 0.05$) was observed only for the first case, i.e: $FA_{Control} > FA_{Patient}$. When correcting for age there were significant decreases in FA in the white matter bundles

of the anterior corpus callosum in MDD subjects compared to controls (Figure 1). There was no significant correlation with the significant voxels and either HAMD or the Beck Depression Inventory in the MDD group. Figure 1: mean FA map (in gray scale), mean FA skeleton (in green), significant voxels ($p < 0.05$) in orange-red.

Discussion: TBSS allows for the unbiased voxel level comparison of study population while circumventing many of the limitations associated with both region of interest and whole brain voxel level analyses. The white matter abnormalities observed are consistent with a large body of literature suggesting disruptions in the connectivity between cortical and subcortical structures in MDD. The abnormalities could be related to both the emotional and cognitive difficulties associated with the illness. The abnormalities were more widespread when age was not included in the model. As this was a much younger group on average compared to late-onset depression studies, it is unlikely these abnormalities were related to vascular changes associated with aging. There was no correlation between FA and either subjective or objective depression suggesting this is a trait abnormality. Future work will examine the cortical and subcortical areas associated with altered white matter tracts in the anterior corpus callosum.

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134. White Matter Alterations in Youth at High-Risk for Bipolar Disorder

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Background: Previous neuroimaging studies have found white matter (WM) structural differences between youth with bipolar disorder (BD) and healthy controls (HC). To examine whether these abnormalities predate the onset of mania, we examined white matter structure in youth at high-risk for bipolar disorder, before their first putative manic episode, using diffusion tensor imaging (DTI).

Methods: *Participants* Fifteen children at high risk (HR) for BD from 9-19 years old and 16 gender and age matched healthy controls (HC) were recruited from the community. The HR group was defined as having at least one biological parent with BD I or BD II, as defined by DSM-IV-TR criteria, and having ADHD with at least moderate mood symptoms and/or a history of a major depressive episode. HC had no current or lifetime psychiatric disorder, nor any first degree relative with a DSM-IV psychiatric diagnosis.

Image acquisition and analysis Magnetic resonance images were acquired using a GE-Signa 3-Tesla scanner using a DTI sequence with 64 slices in 60 diffusion directions. DTIstudio (https://www.mristudio.org) was used to create Fractional Anisotropy (FA), axial diffusivity (AD, a quantitative measure of diffusion along the same direction in a white matter voxel), and Radial Diffusivity (RD) images based on the respective calculated FA, AD, and RD for each voxel. We conducted a whole-brain, region of interest (ROI), atlas-based analysis of diffusion-weighted data using Diffeomap v1.6 (implemented in www.mristudio.org). Diffeomap non-linearly registers images to a pre-segmented white matter atlas using a highly elastic algorithm employing dual-contrast Large Deformation Diffeomorphic Metric Mapping. Brain regions were grouped together into the following functional domains for between group comparisons of DTI measures: Executive function (superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, superior parietal lobule, caudate), Social-emotional function (superior temporal gyrus, lateral fronto-orbital gyrus, middle fronto-orbital gyrus, gyrus rectus, insula, amygdala), Sensorimotor function (pre-central gyrus, post-central gyrus, corticospinal tract, anterior limb of the internal capsule, posterior limb of the internal capsule (PLIC), Visual function (cuneus, lingual gyrus, fusiform gyrus, superior occipital gyrus, inferior occipital gyrus, middle occipital gyrus, superior longitudinal fasciculus (SLF), sagittal stratum (SS), tapetum), and Memory (hippocampus and parahippocampal gyrus). The corpus callosum was also examined. Multivariate analysis of covariance (MANCOVA, covarying for age and IQ) was performed for between group comparisons using SPSS v18. ANCOVA's were performed for individual regions comprising a dependent variable set when overall models were significant or approached significance.

Results: Preliminary results show a main effect of group in the overall MANCOVA model for AD within left visual regions ($p = 0.01$), which appeared to be due to significant AD reduction in the left SS in the HR group ($p = 0.004$). A statistical trend was observed for FA within left sensorimotor regions ($p = 0.09$), due to increased FA in the left PLIC ($p = 0.007$) of the HR group. Finally, there was a statistical trend for the right visual regions model for RD ($p = 0.09$), due to decreased RD within the right SLF in the HR group ($p = 0.01$).

Discussion: We found white matter abnormalities in youth at high-risk for BD using Diffeomap, a novel method for DTI analysis, not yet utilized in BD studies.Youth at HR for BD showed WM aberrations in the left SS, the left PLIC, and the right SLF. The SS is a complex fiber bundle comprising the inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus, and portions of the SLF, splenium of the CC, and optic radiation. The SLF connects the PFC to the parietal cortex and is responsible for visual spatial attention. These WM tracts have been shown to have differences from HC in studies of both HR (ILF) and youth with BD. Sensory, optic radiation, and auditory radiation fibers pass through the PLIC. The SS connects the frontal cortices, regions responsible for executive functioning and attention, to occipital lobes, or areas responsible for visual processing. Individuals with BD have executive functioning and attention difficulties, as well as visual sensory processing deficits, which might be reflected as WM aberrations in the SS, PLIC, and SLF. Disrupted WM architecture could lead to functional disturbances in network connectivity and mood dysregulation. Our findings suggest that WM tract disruptions may precede the onset of mania in high-risk youth.

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135. Ictal Perfusion SPECT reveals Focal Seizures associated aith a New Form of ECT: Focal Electrically-Administered Seizure Therapy [FEAST]

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Background: Imaging studies suggest that physiological alterations in distinct brain circuits are associated with the therapeutic and

adverse cognitive effects of electroconvulsive therapy (ECT). Reduced perfusion and metabolism in prefrontal cortex (PFC) regions has been repeatedly linked to superior clinical outcome. In contrast, and, although less well studied, there is evidence that the magnitude of anterograde and retrograde amnesia correlates with reductions in functional activity of temporal lobe structures. Focal Electrically Administered Seizure Therapy (FEAST) uses a new electrode positioning, electrode geometry and unidirectional stimulation. FEAST theoretically initiates seizures focally and specifically in the prefrontal cortex prior to secondary generalization. FEAST has shown promising clinical results in an open-label study (data presented in a separate poster at ACNP).
We hypothesized that if FEAST induced seizures were more focal and initially limited to the right prefrontal cortex (as seen in computer models and primate studies), then ictal single photon emission computerized tomography (SPECT) injections during FEAST would show different areas of change compared to other conventional forms of ECT. We hypothesized that FEAST-induced seizures would show greater focality in the PFC by increased activity during ictal induction when compared to ultra-brief right unilateral (UB RUL) or bilateral (BL) ECT.
Methods: Unmedicated depressed adults referred to ECT received FEAST, UB RUL or BL treatments. Each underwent 2 SPECT scans (baseline and early ictal period) with an injection of 30 mCi (1,110 Mbq) of technetium-99 bicisate (ECD; Neurolite, DuPont Pharma) followed by an uptake scan 30 minutes later using a triple-headed Picker camera. *SPECT baseline:* One to 3 days prior to any ECT, the radiotracer was injected intravenously following a 15-minute rest period during which subjects sat in a dark, quiet room with their eyes closed. Subjects rested for an additional 15 minutes before scan acquisition. *SPECT early ictal:* Scanning took place on the second session of the ECT course. The bolus tracer was injected in the ECT suite, 12 seconds prior to treatment administration (an 8 seconds stimulation train). After 20-30 minutes of normal recovery, patients were escorted to nuclear medicine for scan acquisition. Data Analysis: Differences in rCBF across both conditions and all three groups were compared using voxel by voxel image analysis with statistical parametric mapping (SPM8, Institute of Neurology, London, UK). First, images were spatially co-registered to an individual high resolution structural MRI scan, then normalized using SPM8 template and smoothed by a Gaussian kernel of 12 mm FWHM. Global normalization of rCBF was done and relative gray-matter threshold was set to 0.5. For each ECT modality, images were compared to baseline using a paired t-test.
Results: To date, 11 patients completed both baseline and corresponding early ictal scans (FEAST = 7, UB RUL = 3, BL = 1; mean age 51 ± 17; 4 males; 1 bipolar disorder). Preliminary results indicate overall increased activity in bilateral prefrontal, medial temporal lobes and pre-motor areas during early ictal conditions compared to baseline. More specifically, the FEAST ictal scans, compared to baseline, are associated with relatively more focal activation in the right prefrontal and pre-motor areas (underneath the large electrode). The imaging study is ongoing and final data (7 in each group) will be presented at the poster session.
Discussion: These SPECT scans suggest that FEAST induced seizures are more focal than conventional RUL and BL ECT. Inter-ictal and post-ictal SPECT scans offer a means to test optimal FEAST parameters and placement, including the impact of reversing current directionality on seizure expression, further refining this new approach to ECT.
Disclosure: E. Short: None. **K. Spicer:** None. **C. Burns:** None. **M. Archer:** None. **M. Schmidt:** None. **H. Sackeim:** Part 1: Consultant, MECTA Corporation. **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. **Z. Nahas:** Part 1: Neuronetics Inc (unpaid), Cyberonics Inc. (past), Avanir Pharmaceutical (past), Aventis Pharmaceutical (past), Neuropace (unpaid) Speaker Bureau: Neuronetics Inc (past), Cyberonics Inc. (past), Part 4: National Institute of Mental Health (NIMH), National Alliance of Research on Schizophrenia and Depression (NARSAD), Hope for Depression Research Foundation (HDRF), Neuronetics Inc (past), Cyberonics Inc, Medtronic Inc., MECTA, Eli Lilly (past), Integra (past).

136. Longitudinal Amygdalar Neuroanatomy in Adolescents with Bipolar I Disorder

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Background: Structural magnetic resonance imaging studies of children and adolescents bipolar disorder (BD) have consistently demonstrated volumetric differences in the amygdala, which is important in the regulation of emotion. However, it remains unclear whether such changes occur close to the onset of illness, and progress with the progression of mood symptoms. The goal of this study was toexamine volumetric differences in the amygdala in adolescents who recently experienced their first episode of mania and 12 months after a baseline scan to identifypossible neuroanatomical changes in this region that may be found early in the course of BD.
Methods: Adolescents (13-18 years old) who were diagnosed as having their first episode of fully syndromal bipolar I disorder within the past 9 months ($N = 21$) and adolescents without any personal or family history of a DSM-IV Axis I disorder ($N = 20$) were examined for group differences in amygdala volumes after collection of high-resolution magnetic resonance structural images using a 3T GE scanner at a baseline and at a 12 month follow up visit. Diagnoses were determined by the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (Geller *et al.* 1996), with established interrater reliability (kappa > 0.9). Manual tracing morphometric analyses were performed using Brain Image Java software (http://spnl.stanford.edu/tools/brainimagej.htm) by a rater blind to group status. SPM8 was used to segment into grey, white, and CSF partitions, and to measure total brain volumes (TBV).
Results: Relative to controls, adolescents with BD showed no significant changes in right, left, or bilateral amygdala volumes after covarying for total brain volume, age, gender, and IQ ($p > 0.05$). At baseline, relative to controls, adolescents with BD showed significant increases in right amygdala volumes after covarying for total brain volume, gender, and age ($F(47) = 6.46$, $p = 0.01$). Right amygdala volume within the BD group correlated positively with higher total ($R = 0.51$, $p = 0.008$), grey ($R = 0.46$, $p = 0.02$) and white ($R = 0.50$, $p = 0.01$) matter volumes. A trend for a positive correlation between increased clinical global functioning and increased right amygdala volumes ($R = 0.35$, $p = 0.078$) was found. Symptoms of mania, depression, or exposure to lithium did not appear to correlate with any volumetric findings within either group. Youth with BD did not report additional manic episodes at the time of 12-month follow-up.
Discussion: Among the most consistent neuroanatomical findings in pediatric BD is a reduction in amygdalar volume. This may not be seen in youth with BD-I until symptoms have progressed well beyond the initial manic episode, or requires exposure to multiple lifetime manic episodes. Amygdalar enlargement was seen in adolescents early in the onset of bipolar I mania, and appeared to be positively correlated with higher overall functioning. Enlargement in amygdalar volume early in the course of illness in this

study may reflect abnormal developmental pruning of medial temporal circuits, or a compensatory neuroprotective mechanism. Correlations to mood symptom ratings, and effects of medication exposure were considered and appeared to have limited impact on volumetric findings. Future longitudinal analyses after an extended period of time are needed to examine the consequences of volumetric differences in adolescents with BD-I on long-term clinical outcome.

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137. Abnormal Hypothalamus Functional Connectivity in Psychotic Major Depression

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Background: Individuals with major depression that also includes psychotic features (PMD), often present with a neurophysiological and HPA activity profile that is distinct from other depressed patients. The hypothalamus functions both as a neural and an endocrine structure and is a key driver and regulator of HPA activity. In the current study we examined low frequency (.008-0.1 Hz) spontaneous oscillations originating in the hypothalamus to determine if the strength of functional connectivity between the hypothalamus and other brain regions differed in patients with PMD. We hypothesized that disturbed connectivity between the hypothalamus and ventral medial structures proximal to the subgenual cingulate cortex would be present in patients with PMD and that the magnitude of this disturbance would predict psychotic symptoms, and abnormalities in the circadian cortisol rhythm.

Methods: 5 minutes of resting state functional magnetic resonance imaging (fMRI) blood oxygen level dependant (BOLD) signal was collected from 99 subjects in 3 groups, patients with major depression with psychotic features (PMD, $N = 23$), patients with major depression without psychotic features (MDD, $N = 38$) and healthy control subjects (HC, $N = 38$). Each subject underwent a comprehensive psychiatric assessment and 24 hour circadian cortisol monitoring as part of a larger protocol. Each of the fMRI images collected were slice-time and homogeneity corrected, co-registered, normalized to a standard template and smoothed with a 5mm Gaussian kernel. The time series was then de-trended using a voxel-wise global signal correction (linear model for global signal) and band-pass filtered to isolate typical functional connectivity frequency ranges (.008-.1 Hz) from high/low frequency noise. A series of 143 anatomy-based regions of interest (ROI) were defined in normalized space. The average functional time series of the BOLD signal was extracted from each of these ROIs. These extracted time series were then correlated using Spearman's rho. Spearman's rho values for each unique ROI pairing with the hypothalamus (142 in total) was then converted to a Z score using Fisher's R to Z transformation. A factorial multivariate analysis of variance (MANOVA) was then used to determine group differences in connectivity. An a priori significance criteria ($p < 0.05$) was applied to ROIs incorporating portions of the subgenual cingulate (BA25, subcallosal gyrus). A follow-up exploratory analysis was also conducted within the factorial MANOVA model.

Results: Overall multivariate group differences in hypothalamus connectivity were observed ($F = 8.09$, $p = 0.026$). This multivariate finding was a product of omnibus group differences observed between the hypothalamus and the following connectivity partner regions: claustrum, fastigium, inferior orbital cortex, medial orbital cortex, Heschl's gyrus, insula, lentiform nucleus, middle temporal gyrus, olfactory cortex, globus pallidus, pons, putamen, subcallosal gyrus, superior temporal gyrus, superior temporal poles, BA9, BA13, BA18, BA19, BA21, BA22, BA28.

Amongst our a priori ROIs, PMD patients had a significantly lower level of connectivity than HC ($T(59) = 2.603$, $p = 0.012$) and MDD ($T(59) = 2.876$, $p = 0.006$) between the subcallosal gyrus and the hypothalamus. However, the subcallosal connectivity observed in PMD patients was not significantly correlated with depression symptom severity (Hamilton Depression ratings scale total score or Thace endogenous sub-scale), psychotic symptoms (Brief Psychiatric Ratings Scale total score or Positive symptom sub-scale), or overnight cortisol secretion.

Other brain regions with significant omnibus group differences failed to meet the post-hoc Bonferroni corrected significance threshold.

Discussion: Hypothalamus connectivity differences in patients with PMD may be reflective of degraded hypothalamic communication with the rest of the brain. This degraded communication may be a component of the actual disease state, or a reaction/adaptation to the disease processes. While our findings suggest that degraded hypothalamus connectivity to the subcallosal gyrus may be PMD specific, these changes do not appear to be linearly related to observed severity of symptoms or cortisol activity in PMD.

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138. Abnormal Temporal Lobe White Matter as a Biomarker for Genetic Risk of Bipolar Disorder

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Background: Brain white matter abnormalities have been hypothesized to play a role in the neurobiology of bipolar disorder. The nature of these white matter abnormalities is not well characterized, however, and it is unknown whether they occur following disease onset or represent potential markers of genetic risk. In this study we thus examined the brain white matter using diffusion tensor imaging in patients with bipolar disorder, unaffected siblings of patients and healthy volunteers to identify potential white matter biomarkers of genetic risk. We hypothesized that white matter abnormalities identified in patients with bipolar disorder would also be evident in unaffected siblings compared to healthy volunteers.

Methods: We included 26 (11M/15F) patients with bipolar disorder (mean age = 40.6, SD = 12.4), 15 (6M/9F) unaffected siblings (mean age = 42.0, SD = 11.7) of patients with bipolar disorder and 27 (15M/12F) healthy volunteers (mean age = 40.8, SD = 12.5). All subjects received a diffusion tensor imaging exam on a 3T system that included volumes with diffusion gradients applied along 31 non-parallel directions and five volumes without diffusion weighting. Image processing was conducted using the tract-based spatial statistics program within FSL. A nonparametric voxelwise ANCOVA with subject-type (patients, unaffected siblings, and healthy volunteers) as the between-subjects factor and age as a covariate was carried out using permutation statistics via the Randomise tool in FSL with strict family-wise error correction and threshold-free cluster enhancement.

Results: Examination of fractional anisotropy across the entire white matter skeleton revealed three regions in the right temporal lobe that differed significantly ($p < .05$; family-wise error corrected) among the 3 groups. Post-hoc analyses indicated that unaffected siblings had fractional anisotropy that was intermediate to and significantly ($p < .05$) different from healthy volunteers and patients with bipolar disorder across the average of these 3 regions (healthy controls > unaffected siblings > bipolar disorder). Using the three clusters of white matter that differed significantly among groups as seed regions probabilistic tractography indicated that they lie along the inferior frontal occipital fasciculus, a large intrahemispheric association pathway that connects the orbital frontal, temporal, and occipital lobes.
Discussion: Our results suggest that lower white matter integrity in the right temporal lobe may be a biomarker for genetic risk of bipolar disorder. The abnormal white matter regions identified in patients and siblings lie along a major white matter tract that interconnect brain structures strongly implicated in emotion regulation. It is conceivable that the attenuated nature of these white matter abnormalities present in siblings allow for some preservation of adaptive emotional regulation, whereas the more pronounced alterations observed in patients is related to the marked emotional dysregulation characteristic of bipolar disorder. Further work is required to elucidate both the genetic mechanisms underlying right temporal white matter integrity in bipolar disorder as well as the functional implications of this abnormality.
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139. Changes in Brain Activation Following Family-Focused Treatment in Youth at-risk for Bipolar Disorder

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Background: Subthreshold forms of bipolar disorder (BD), such as BD NOS, cyclothymia, and MDD with family history of BD, affect 3 - 9% of youth (Birmaher *et al.*, 2009 ; Miklowitz *et al.*, 2008). Without intervention, these youth are at high risk for progressing to full BD. We recently demonstrated that a 12-week adaptation of Family Focused Therapy for youth at high risk for BD (FFT-HR) reduced symptoms of depression and mania (Miklowitz *et al.*, 2011). We therefore conducted a randomized trial of FFT-HR in 40 at-risk youth, and conducted fMRI pre- and post-treatment in a subset of patients to study the neural correlates and predictors of clinical response. Because our previous studies found that clinical improvement was associated with decreased amygdalar activation and increased DLPFC activation (Chang *et al.*, 2008; 2009), we hypothesized that improvements in symptom severity would be accompanied by changes in activation of the amygdala and prefrontal cortex.
Methods: Subjects: Twelve subjects at risk for BD were scanned (8 male; mean age = 14.1 yrs). Each participant was 9 to 17 years old, had at least one first-degree relative with bipolar I disorder (confirmed by SCID interview), and had either BD-NOS, cyclothymia, or major depressive disorder (by WASH-U-KSADS), and active depressive (CDRS-R > 29) or manic (YMRS > 11) symptoms in the last 2 weeks.
Treatment: 6 subjects received FFT and 6 received TAU (one feedback session and up to 3 crisis sessions).
MRI Acquisition: Subjects were scanned on a 3T GE Signa scanner using a custom-built fMRI head coil. Thirty axial slices (4mm thick, .05 mm skip), parallel to the axis of anterior and posterior commisures, covering the entire brain (FOV = 20cm, 64x64 matrix, in-plane spatial resolution = 3.4 mm) were acquired using a spiral pulse sequence with the following parameters: TR = 2000 msec, TE = 30 msec, flip angle = 80 degrees and one interleave.
Faces Task: Subjects performed a gender identification task while viewing blocks of fearful, calm, and neutral faces, and scrambled images. There were 4 blocks for each condition. Each block included 8 pictures. Each picture was presented for 3 sec, with no inter-stimulus interval.
Data Analysis: Functional data were analyzed using SPM8. FMRI images were reconstructed, spatially realigned to the third image, analyzed and repaired for motion, high-pass filtered, and spatially normalized into standard stereotactic space using the individuals' anatomical scan and an age-appropriate group template (CCHMC) and smoothed with a 7 mm Gaussian filter. Fearful minus scrabmbled faces was the main contrast used. Group analyses used a repeated measures ANOVA in SPM8 to identify clusters of activation that changed significantly from baseline to follow-up. A dual threshold of $p = 0.01$ height and $k = 40$ cluster extent was used. Activation clusters were localized using roimod2 spm toolbox, then superimposed on a single-subject high-resolution T1-weighted image to verify neuroanatomical locations. Mean activation in clusters that fell in apriori hypothesized regions were extracted to SPSS. In SPSS, Spearman's correlations were conducted between activation and CDRS and YMRS scores.
Results: CDRS and YMRS scores decreased significantly following treatment ($p = 0.007$), but not differentially by treatment group ($p = 0.53$). For all subjects, whole-brain analysis showed that activation in the right amygdala declined and in the right DLPFC increased significantly from baseline to follow-up. Greater activation in the amygdala at baseline was associated with greater improvement in depression severity following treatment by FFT, but not TAU ($p = 0.001$; Figure 1). For both treatment groups, greater increases in DLPFC from baseline to follow-up was associated with greater improvement in mania severity following treatment ($p = 0.02$). Two of the subjects in the FFT group and four in the TAU groups were taking medications, which had no discernible effect on amygdala or DLPFC activations.
Discussion: This is the first study to demonstrate neural predictors of response to family therapy in youth at high-risk for BD. Decreased amygdala activation following both active and comparator treatments may reflect overall improved regulation of amygdala reactivity to emotion-related stimuli. Greater activation in the amygdala at baseline may predict better response to FFT. Increased DLPFC activation following both treatments may reflect improved executive control over emotional responses. Limitations of this study include the small sample size and medications taken by the subjects. Future studies with larger samples are needed to confirm these findings and explore further neural mechanisms of response to psychotherapy in this population.
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140. Hippocampal Activation predicts Treatment Response to Antidepressant for Depression

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Background: Although many studies investigated the relationship between hippocampal volume loss and treatment response to antidepressant in depression, no studies have investigated the

capacity for neural responses during memory encoding task to predict treatment response.
Methods: Functional magnetic resonance imaging (fMRI) responses of the brain were examined in individuals with depression (n = 12) versus controls (n = 12). fMRI was examined during memory encoding task adapted for a 1.5T scanner. Patients then received antidepressant treatment. Treatment response was assessed 6months after therapy began.
Results: Prior to treatment and relative to controls, patients exhibited overall increased activity in the right dorsolateral prefrontal, cingulate, insular and occipital cortices. The left hippocampus activation was commonly found in controls and patients. Poor improvement after treatment was associated with smaller hippocampal activation during memory encoding task.
Discussion: Decreased activation within hippocampus during memory encoding task may be a key factor in limiting responses to antidepressant for a certain proportion of depressive patients.
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141. The D_3 Dopamine Receptor in Cocaine Users: PET Studies with [^{11}C]-(+)-PHNO

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Background: Previous positron emission tomography (PET) studies have shown that drug-addiction is associated with lower D_2-type dopamine receptorsin brain. In contrast, some preclinical data suggest that repeated exposure to drugs (e.g.: cocaine) increases expression of the D_3 receptor, a member of the D_2 receptor subfamily. Despite the suspected role of the D_3 receptor in reward-related behaviours and addiction, there are no published data on the status of this receptor in living addicted humans. The objective of this study isto test the hypothesis, via PET measurements of the D_3-preferring agonist ligand [^{11}C]-(+)-PHNO, that unlike the D_2 dopamine receptor, which is reportedly down-regulated in cocaine addiction, D_3 receptor binding would be increased in cocaine users.
Methods: 9 healthy subjects (1F, 8M; mean age 39 yrs) and 8 abstinent cocaine users (1F, 7M; mean age 42 yrs; mean days of abstinence 30, range 7-70) underwent PET scanning following [^{11}C]-(+)-PHNO and for comparison, following the administration of the mixed $D_{2/3}$ receptor antagonist [^{11}C]raclopride. [^{11}C]-(+)-PHNO and [^{11}C]raclopride binding (BP_{ND}) were investigated in regions of interest including the dorsal (anterior and posterior caudate and putamen) and ventral striatum, the globus pallidus, and the substantia nigra, using the simplified reference tissue model. The ratio of D_3 to D_2 binding in brain was estimated by calculating [^{11}C]-(+)-PHNO BP_{ND} in brain areas where binding has been shown to be exclusive to D_3 (substantia nigra) and D_2 receptors (dorsal caudate).
Results: Cocaine use was associated with lower [^{11}C]-(+)-PHNO binding (~10-12%, maximal in the dorsal caudate, $p = 0.03$) and [^{11}C]raclopride binding (~12-20%, maximal in the dorsal caudate, $p = 0.09$) in the D_2-rich dorsal striatum. In the substantia nigra, cocaine use was associated with greater [^{11}C]-(+)-PHNO binding (~13% (NS)) in individuals who had recently used cocaine (n = 5, mean days of abstinence ~7-10 days; compared to individuals >2mo abstinent), but not in the group as a whole (~4%). Relative to control subjects, the ratio of D_3 to D_2 binding ([^{11}C]-(+)-PHNO BP_{ND} in substantia nigra / dorsal caudate) was 17% greater in cocaine users. [^{11}C]-(+)-PHNO and [^{11}C]raclopride binding were significantly correlated (r = ~0.6-0.7) in striatum (but not in substantia nigra or globus pallidus). [^{11}C]-(+)-PHNO binding in the dorsal striatum (dorsal putamen) was correlated with drug abuse severity (r = ~0.7).
Discussion: Adding to the current PET/[^{11}C]raclopride literature suggesting lower $D_{2/3}$ receptor density in addiction, these preliminary [^{11}C]-(+)-PHNO data suggest that the D_3 receptor might be selectively up-regulated by repeated cocaine use. This finding supports our previous finding of increased D_3 receptor binding in methamphetamine users (unpublished), and suggests that greater D_3 activity might play a role in addiction.
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142. Up-Regulation of Nicotinic Acetylcholine Receptors in Menthol Cigarette Smokers

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Background: A third of cigarette smokers use predominantly menthol-flavored cigarettes. Compared to non-menthol cigarettes, menthol cigarettes lead to elevated serum nicotine levels and more difficulty quitting in standard treatment programs. Previous brain imaging studies of humans smokers demonstrate that smoking in general (without regard to cigarette type) leads to up-regulation of â$_2$-containing nicotinic acetylcholine receptors (nAChRs) compared to non-smokers and former smokers. We sought to verify up-regulation of nAChRs in smokers and to determine whether menthol cigarette usage results in greater nAChR up-regulation than non-menthol cigarette usage.
Methods: 114 participants (n = 41 non-menthol cigarette smokers, n = 22 menthol cigarette smokers, and n = 51 non-smokers) underwent positron emission tomography (PET) scanning, using the radiotracer 2-[^{18}F]fluoro-A-85380 (2-FA). 2-FA specific binding volume of distribution (V_S/f_P) was determined for five brain regions (thalamus, brainstem, cerebellum, prefrontal cortex, and corpus callosum), as measures of $\alpha_4\beta_2^*$ nAChR density.
Results: An overall test of V_S/f_P values across all brain regions revealed a between-group (smoker versus non-smoker) difference (MANCOVA; df = 5, 107; F = 20.4; P < 0.0005). Follow-up tests for individual brain regions revealed significant differences between groups for the prefrontal cortex, brainstem, cerebellum, and corpus callosum (ANCOVAs, all df's = 1, 113; F's = 15.0, 21.5, 21.1, and 8.9; P's all < 0.0005, except for corpus callosum, where P < 0.005), but not for the thalamus (ANCOVA; df = 1, 114; F = 0.2, n.s.). For regions found significant in the preceding analysis, $\alpha_4\beta_2^*$ nAChR levels were 36 to 42% higher for smokers than non-smokers. In comparing menthol versus non-menthol smokers, the overall test revealed a significant between-group (menthol versus non-menthol) difference, (MANCOVA; df = 5, 56; F = 2.9; P < 0.05). Follow-up tests for individual brain regions revealed significant between-group differences for the brainstem, cerebellum, and corpus callosum (ANCOVAs, all df's = 1, 62; F's = 7.5, 9.2, and 7.5; all P's < 0.01), with menthol smokers having 21 to 28% higher nAChR density in these regions. Exploratory tests also revealed a significant decrease in nAChR density with increasing

age across the total study sample (MANCOVA; df = 5, 107; F = 7.4; p < 0.0005).
Discussion: Nicotine exposure appears to be a primary determinant of nAChR up-regulation. Therefore, more severe up-regulation of nAChRs in menthol cigarette smokers suggests higher brain nicotine exposure in this group. Study results provide additional information about the severity of menthol cigarette use, and may explain why these smokers have more trouble quitting in standard treatment programs.
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143. 16 msec "Unseen" Cocaine and Sexual Cues Recruit Limbic Motivational Circuitry

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Background: In 2008, our laboratory provided the first evidence that "unseen" 33 msec drug and sexual cues presented entirely outside awareness can activate reward-relevant brain circuitry (amygdala, ventral striatum/ventral pallidum; orbitofrontal cortex/ventral prefrontal cortex, insula, temporal pole) in cocaine patients (PLoS ONE, 2008). Importantly, a heightened brain response to these "unseen" appetitive cues was associated with rapid (vs. delayed) relapse (CPDD, 2011) in this same population – underscoring the motivational significance of these rapid, unconscious brain responses. A software malfunction recently gave us the unexpected opportunity to assess whether (even) "unseen" 16 msec cues can recruit reward-relevant circuits, offering a unique "early" window onto the development and expression of unconscious appetitive motivation.
Methods: We used randomized, event-related BOLD (Blood Oxygen-Level-Dependent) fMRI (functional magnetic resonance imaging) at 3 Tesla to measure the brain response to cocaine-related, appetitive (sexual), aversive (bodily injury or disease), or neutral cues of 16 msec duration (each "backward-masked" by a 467 msec neutral stimulus) in chronic cocaine users (n = 9, ongoing). The 16 msec duration resulted from an E-Prime software malfunction causing masks to systematically appear ~ 17 msec prior to their scheduled occurrence, eclipsing half of the intended (33 msec) target duration. Stimuli (24 unique stimuli presented twice, in each of the 4 cue categories, and 48 null events) were "jittered" in order to optimize coverage of the hemodynamic response function (HRF). Data were smoothed, normalized, realigned and batch-analyzed within SPM 5, using HRF as the basis function. Pre-planned contrasts compared the brain response to evocative (cocaine, sexual, aversive) vs. neutral cues.
Results: "Unseen" 16 msec cocaine and sexual cues recruited limbic motivational circuitry (e.g., ventral striatum/pallidum/BNST/dorsal amygdala; brainstem; p < 0.005 uncorrected; 2< t< 5). The "unseen" aversive cues lacked this recruitment pattern. Intriguingly, the differential brain response to "unseen" 16 msec cocaine and sexual cues was not detectable in the first 24 trials, but emerged robustly in the second half of the task, after repeated presentations.
Discussion: To our knowledge, this is the first demonstration that fMRI (by the use of 'cognitive subtraction') can reliably detect the brain impact of "unseen" 16 msec cues with motivational relevance. The finding underscores the sensitivity of our patients' brain motivational circuitry to appetitive (drug and sexual) cues, even when these stimuli are well below the threshold for conscious recognition. The emergent, "recruiting" pattern of limbic activation revealed by the 16 msec paradigm suggests that our cocaine patients' motivational circuits may 'kindle' with repeated cocaine cue exposure. A 'kindling-like' phenomenon could help to explain 1) the pathognomonic persistence of cue-triggered drug motivation in addiction, 2) the familiar resistence of cue-triggered responses to Pavlovian extinction (non-reinforced exposure), and 3) the preliminary efficacy of "anti-seizure" medications (e.g., gamma vinyl gaba; topiramate) in cocaine dependence.
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144. Neural Responses to Acute IV Alcohol in Healthy Moderate Drinkers

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Background: Alcohol is one of the most commonly consumed psychoactive substances in the USA, yet despite its widespread use and the wealth of alcohol research, the neuroanatomical targets of acute alcohol doses in humans remain unclear. A better understanding of these targets may explain why some people drink alcohol excessively and develop abuse problems, whereas others do not. Also, individual differences in alcohol subjective experiences may influence alcoholism risk. For example, individuals who report positive stimulant-like effects (i.e., energized) also report greater drug liking and consume more alcohol than those who do not experience stimulation. In this study we used functional magnetic resonance imaging techniques to measure real-time changes in brain activity and subjective experience after an intravenous (IV) dose of alcohol.
Methods: Healthy men and women participated in three experimental sessions. The first session was a laboratory session with single-blind IV administration of 6% alcohol delivered by a pump to determine individualised infusion profiles for each subject to achieve a breath alcohol concentration of 80mg%. The next two sessions involved an fMRI scan (Phillips 3T Achieva Quasar scanner) with a double-blind IV infusion of alcohol or placebo in randomised order administered using the infusion profile measured during the first session. fMRI BOLD data and real-time subjective ratings of mood and drug effects were acquired during the 50min scan. Imaging data were analysed using non-linear regression and a difference of exponentials signal model reflecting the pharmacokinetics of drug-brain interactions.Data were corrected for multiple comparisons at p<0.05 via Monte Carlo simulation.
Results: Overall, alcohol increased subjective stimulation, sedation and ratings of feel drug effects, like drug, and want more drug. Preliminary data (N=10) indicates that, in comparison to placebo, alcohol significantly increased BOLD response in the left middle frontal gyrus, the left subcallosal gyrus and nucleus accumbens area, and the right parahippocampal region and amygdala,. Ratings of drug liking were positively correlated with responses in the right parahippocampal gyrus-amygdala and the left medial frontal gyrus. Ratings of stimulation and sedation after alcohol were negatively correlated with responses in the lentiform nucleus. Furthermore, responses in this area were significantly lower among subjects who experienced stimulant-like effects after alcohol than those who did not experience stimulation after alcohol.

Discussion: These findings demonstrate that dynamic alcohol-induced changes in BOLD signal can be detected using a pharmacokinetic model-based analysis strategy without the need for performance-induced activation. They show that alcohol directly activates mesolimbic and mesocortical dopaminergic projections which regulate learning, memory and motivation, and they suggest that individual differences in the subjective effects of alcohol correspond with differences in brain activity. These data provide a basis for future investigations of the direct effects of alcohol upon the brain which can help us to elucidate sources of individual difference in susceptibility to alcohol use disorders.
This research is supported by NIAAA R21 DA20716 and [in part] by the Intramural Research Program of the NIH, NIDA.
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145. Denicotinized vs Average Nicotine Tobacco Smoking differentially Releases Striatal Dopamine

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Background: Tobacco dependence involves pharmacological, neurophysiological, and psychological factors. Nicotine has long been recognized as a necessary but an insufficient component of tobacco cigarettes to maintain a psychopathologic need to smoke. This study examined the importance of nicotine in cigarette smoking after overnight abstinence to release striatal dopamine (DA).
Methods: Twenty four male smokers were selected to smoke either denicotinized (denic) or average nicotine (avnic) cigarettes under single blind conditions. Six male nonsmokers were selected for sham smoking controls. All were given [^{11}C]raclopride and scanned in a PET facility. Mood scales as well as venous plasma nicotine were determined before and after smoking.
Results: Smoking either denic or avnic cigarettes released striatal DA differentially compared to sham controls. Denic cigarette smoking released DA primarily in the right striatum, whereas avnic smoking released DA in both striata but especially on the left. Increases in venous plasma nicotine concentrations correlated positively with increased DA release in the left caudate nucleus. Smoking denic cigarettes reduced craving as much as smoking avnic cigarettes. Craving reduction after avnic smoking correlated negatively with increased plasma nicotine.
Discussion: Although smoking denic and avnic cigarettes reduce craving equally, they produce different regional brain release of DA, the former primarily in the right, and the latter in the basal ganglia of both cerebral hemispheres.
Disclosure: E. Domino: None. **L. Ni:** None. **J. Domino:** None. **W. Yang:** None. **C. Evans:** None. **S. Guthrie:** None. **R. Koeppe:** None. **J. Zubieta:** None.

146. DAT Genotype-Dependent Baclofen-Induced Inhibition of Ventral Striatum and Medial Orbitofrontal Cortex Brain Responses to Smoking Cues: Moving Toward a Personalized Medicine Approach to Cigarette Addiction

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Background: Cigarette addiction is the leading cause of preventable death in our nation. Despite the life-threatening health consequences of smoking and the substantial heavy economic burden on society, close to 25% of the population continues to smoke. Two major factors contribute to continued smoking and relapse: craving elicited by smoking cues (SCs) and craving elicited by nicotine withdrawal (WD).Inability to combat WD-induced craving, which declines within a month, plays a major role in early relapse. However, smokers report that SCs can trigger relapse months or even years after quitting. Existing smoking cessation medications focus on alleviating WD and/or blocking nicotine reward, and are helpful for subgroups of smokers. However, other 'cue-vulnerable' smokers have less success. Thus, there is a critical need to identify agents that can improve treatment outcome in SC-vulnerable individuals. A number of factors, including genetic variance, may underlie the relative contribution of SCs and WD to the maintenance of dependence and to relapse. Indeed, using perfusion fMRI and evocative SCs, we found (Franklin *et al* NPP '09), and confirmed (Franklin *et al* Addiction '11), a profound effect of variance in the dopamine transporter (DAT) gene on brain responses during SC exposure: smokers carrying a 9-repeat allele had robust responses in the reward-relevant ventral striatum and medial orbitofrontal cortex (VS/mOFC) while homozygotes for the 10-repeat had little or no brain responses in these regions. GABA B agonists modulate dopamine and have shown promise as drug cue blocking agents. The GABA B agonist, baclofen, has shown promise in treating alcohol, cocaine, methamphetamine, opiate, and cigarette addictions. We demonstrated that it reduced the number of cigarettes per day in a smoking reduction clinical trial (Franklin *et al* DAD 2009), and, that three-weeks chronic baclofen reduces activity in the brain at rest in the VS/mOFC and amygdala in smokers (Franklin *et al* DAD '11). Thus, we hypothesize that baclofen may be an effective agent to aid a SC-vulnerable endophenotype.
Methods: To test our hypothesis, in a within-subject design, we administered either one 20-mg dose of baclofen (onBAC) or no medication (offBAC) to N = 12 nicotine dependent smokers and acquired both resting baseline data and functional data while smokers performed a Craving Modulation Task. We used a BOLD block-design that consisted of six counterbalanced blocks of three 20-second conditions. In two of the conditions, (nonSC and SC) subjects were instructed to just watch the pictures (Watch). In an additional SC condition smokers were instructed to inhibit their craving for a cigarette (Down). Subjects were grouped by DAT genotype and data were analyzed in SPM 8 for Watch SC (-) Watch nonSC and for Watch SC (-) Down SC conditions.
Results: In all subjects, similar to chronic baclofen, acute baclofen blunted VS/mOFC and amygdala activity in the brain at rest, without differences in sedation across conditions. In the Craving Modulation Task, reward-related activity was not different in the on versus offBAC conditions for Watch SC (-) Watch nonSC and no differences were observed in Watch SC (-) Down SC in all subjects. However, analysis by genotype revealed that in 9-repeat carriers only, onBAC versus offBAC responses to Watch SC (-) Watch nonSC were reduced in the VS/mOFC and increased activity in these regions was observed in the Watch SC (-) Down SC, indicating an enhanced ability to inhibit. 10-repeat homozygotes had greater responses in the mOFC in the onBAC versus offBAC condition to Watch SC (-) Watch nonSC and showed no differences in activity to Watch SC – Down SC.
Discussion: These results have important clinical implications as they demonstrate that acute baclofen is effective at blunting limbic circuitry in the brain at rest and, that it enables inhibition of brain responses in reward-related circuitry during SC exposure in a DAT-genotype dependent manner, potentially identifying a pharmaco-responsive endophenotype. Some smokers can control their craving and remain abstinent for months or even years after quitting. Given that relapse may occur long after WD symptoms abate and long-term treatment may not be a practical solution in

9-repeat carrier 'cue vulnerable' smokers, acute baclofen may suffice to immediately block drug-motivated behavior during 'at risk' situations. Ultimately, the goal for contemporary medicine is to establish brain/behavioral/genetic endophenotypes that predict medication response, and foster the development of individualized treatment strategies,helping to conquer a devastating and deadly disease: Cigarette Addiction.
Disclosure: T. Franklin: None. **J. Suh:** None. **J. Shin:** None. **R. Hazan:** None. **K. Jagannathan:** None. **Z. Singer:** None. **Y. Li:** None. **Z. Wang:** None. **M. Goldman:** None. **R. Ehrman:** None. **C. O'Brien:** Part 3: Alkermes. **F. Lohoff:** None. **A. Childress:** None.

147. A Role for Cannabinoid CB1 Receptor Signaling in Social Behavior

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Background: A variety of clinical and preclinical studies show that cannabinoids have a profound effect on stress regulation and emotion. The psychoactive component of the cannabis plant delta-9-tetrahydrocannabinol (THC) binds to the endogenous endocannabinoid system to exert its effects on physiology and behavior. Cannabis has been suggested to produce euphoria and prosocial behavior by binding to endocannabinoid receptors that in turn interact with the hypothalamic-pituitary-adrenal (HPA) stress axis. The endocannabinoid system is a neuroactive lipid signaling system that serves to gate synaptic transmitter release. Substantial evidence implicates this system in the responsiveness to stress and glucocorticoids via actions in the hypothalamus and limbic structures, areas central to the expression of both anxiety-like and affiliative behaviors. In order to test the role of endocannabinoid signaling in social anxiety and memory, we utilized a combined genetic knockout (KO) and pharmacological approach. Specifically, we assessed the effects of a global CB1 receptor KO (CB1-KO) and systemic administration of a CB1 antagonist (5 mg/kg AM251; Tocris) on social anxiety in a social investigation paradigm and social memory in a social discrimination test.
Methods: We assessed the effects of a global CB1-KO and systemic (intraperitoneal, 30 minutes prior to testing) administration of a CB1 antagonist (5 mg/kg AM251; Tocris) on social anxiety in a social investigation paradigm and social memory in a social discrimination test. For this purpose we analyzed spatial preference with respect to conspecific stimuli and ethologically relevant indices of sociality, fear and anxiety.
Results: Results showed that when compared to wildtypes (WT) and vehicle treated animals, CB1-KOs,or animals that received an acute dose of the CB1 receptor antagonist AM251, showed elevated freezing and risk-assessment behaviors in response to a novel male conspecific, consistent with an anxiogenic profile. In the social discrimination test, CB1KOs and animals that received the CB1 antagonist showed improved social memory in the social discrimination test. In addition, CB1KOs spent more time investigating the novel conspecific, while the acute dose of the CB1 antagonist produced substantially low investigation times.
Discussion: These results clearly implicate CB1 in the regulation of social anxiety toward unfamiliar conspecifics. Further, the anxiogenesis resulting from either total CB1 deletion or an acute obstruction may promote enhanced social discrimination/memory.
Disclosure: Y. Litvin: None. **M. Hill:** None. **B. McEwen:** Part 4: small unrestricted grant from Johnson and Johnson Inc. **D. Pfaff:** None.

148. Oxytocin at the Initiation of Romantic Love: Relations to Couple Interactive Reciprocity

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Background: The ability to form enduring and meaningful relationships is a key social capacity of humans. Romantic attachment is among the central forms of attachment in adulthood and has a profound effect on adult life. Satisfying and stable intimate relationships have been associated with physical and psychological health, whereas inability to establish romantic bonds or maintain satisfactory close relationships is linked to physical and emotional distress. Animal research suggests that the neuropeptide Oxytocin is involved in processes of attachment, maternal behavior, and pair bonding. Due to its pro-social and anxiolytic effect, Oxytocin has recently been examined as a potential therapeutic agent in psychiatric conditions associated with disruptions to social bonding, including autism, schizophrenia, depression and post-traumatic stress disorder. However, to our knowledge, no study has examined the role of Oxytocin during the initial stages of romantic attachment. In this study, we assessed new romantic partners during the period of falling in love and six months later in comparison to a matched group of romantically-unattached singles. In addition to assessing plasma Oxytocin level, we examined whether (a). Oxytocin levels are individually stable during the period of pair bonding in humans, (b). Oxytocin levels at the initial period of falling in love can predict whether the couple will stay together six months later, and (c). Oxytocin levels are associated with couples' interactive behavior, similar to the findings reported for parent-infant interactions.
Methods: Two groups of young adults were recruited, including 120 young adults (60 couples) who began a romantic relationship within the past three months (Mean relationship duration = 2.4 months) and 43 romantically unattached matched controls. New lovers who stayed together six months following the initial visit were seen again. Plasma Oxytocin levels were assessed at each stage, lovers were interviewed regarding their relationships and were videotaped during a discussion of a shared positive experience. Interactions were coded using the CIB coding system, a well-validated system for coding dyadic interactions.
Results: (a) During the period of falling in love, Oxytocin levels show a dramatic increase in comparison to singles, $F(1,152) = 109.33$, $p < .001$ and the increase was similar for men and women. Furthermore, among those who stayed together, Oxytocin levels did not drop approximately 8 months after the initiation of the romantic relationship. (b) Approximately a third of the couples did not stay together and we examined whether initial Oxytocin levels during the period of falling in love can differentiate those who did or did not continue the relationship. Significant difference was found in Oxytocin levels of new lovers at the first time-point between those who would stay together six months after the experiment and those who would not, $F(1,97) = 4.67$, $p < .05$. (c) Couples' interactive reciprocity score was significantly correlated with Oxytocin levels at the first ($r = 0.29$, $p < .01$) and second ($r = 0.27$, $p < .05$) time-points. Moreover, the degree of preoccupation in the relationship was also positively correlated with Oxytocin levels at the first time-point ($r = 0.19$, $p < .05$).
Discussion: Oxytocin shows a dramatic increase during the initial period of romantic love and is individually stable across the period of pair bonding in humans. Oxytocin levels at the initial period of falling in love differentiated couples who will stay together six months later. Similar to the findings reported for parent-infant interactions, Oxytocin was associated with couples' reciprocal behavior. Taken together these findings provide further evidence of the Oxytocin's important role in formation and maintenance of close relationships and social communication throughout life.

Disclosure: I. Schneiderman : None. **O. Zagoory-Sharon :** None. **J. Leckman :** None. **R. Feldman :** None.

149. Puberty: A Sensitive Period in the Neurobiology of Stress and Emotion. A Study of Internationally Adopted Youth at the Pubertal Transition

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Background: Puberty is a sensitive period during which rising levels of gonadal steroids reprogram the responsiveness of the startle response and the HPA axis. In addition, pubertal changes influence emotion-processing brain areas including those that regulate the HPA axis and startle responsiveness. In research on children and youth, the onset of puberty has been associated with increases in both basal and stress reactivity of the HPA axis as well as increased activity in defensive biological systems. Basal cortisol levels increase from late childhood through early adolescence, perhaps peaking around Tanner Stage 3. Importantly, adolescents at higher stages of pubertal development show a larger CAR, steeper diurnal decline, more elevated daytime cortisol and increased cortisol responses to stressors. To date, however, it is unknown how adverse early care and puberty interact to influence the activity of the HPA axis or the psychophysiology of defensive and appetitive motivations.
Methods: We assessed the impact of maltreatment on defensive (startle response) and appetitive (post-auricular reflex) emotion processing and the cortisol awakening response (CAR) in pre/early and mid/late pubertal youth survivors of chronic physical and emotional neglect. We studied 12-13 year old youth adopted from orphanages and hospitals (post-institutionalized, PI, N = 55, 8-84 mos at adoption), in contrast to 2 groups: youth adopted from foster care (FC; n = 44, 2-8 mos at adoption), and a non-adopted youth (NA, n = 60).
Results: Neglect: Among adopted youth, severe maltreatment was associated with blunted potentiated startle (STL) and they showed lower overall startle magnitude than NA's. Additionally, PI youth showed a blunted CAR compared to FC and NA youth and severe maltreatment among adopted youth was linked to blunted CAR. However, adopted youth showed larger overall post auricular (PAR) reflex than NA.
Puberty: Pre/early-pubertal adopted youth showed typical STL and PAR potentiation but by mid/late puberty, STL and PAR potentiation were blunted. This was in contrast with patterns exhibited by the NA of lower STL and PAR potentiation before puberty but enhanced potentiation after puberty.
Conclusion: Defensive, appetitive emotions and stress-related preparation for upcoming challenges are all imprinted by early chronic maltreatment. Blunted defensive emotions and atenuated preparation for upcoming challenges (blunted CAR) are sequelae of chronic early maltreatment. However, sensitive developmental periods, in particular pubertal related processes, play a decisive role in how chronic early neglect shapes the neurobiology of stress and emotion.
Discussion: Many years post-adoption, youth adopted from institutional care showed blunted startle response and flatter CAR than youth reared in their families of origin. A similar pattern was noted among the combined group of post-institutionalized and post-foster care youth when parental report of adverse pre-adoption care was examined on CAR and startle potentiation. Nonetheless, puberty moderated these associations for the CAR, and the effects of level of adverse early care were no longer significant for mid/late pubertal youth, suggesting that puberty may open a window for reprogramming of the HPA axis. Additionally, the physiology of emotion processing of adoptees may follow a different trajectory with the onset of puberty compared to their not-adopted peers. Specifically, international adoptees tend to show diminished defensive and appetitive potentiation with advanced puberty in contrast with increased potentiation for not-adopted youth. Overall our results show that the distal impact of disturbed prior developmental tasks will emerge in the context of current environments and new developmental tasks. Specifically, atypical HPA axis function and blunted defensive reacitivty associated with early social and physical neglect is still evident in later years, but the new developmental tasks of puberty in the context of improved psychosocial circumstances modify the impact of early adverse care.
Disclosure: K. Quevedo: None. **A. Johnson:** None. **M. Loman:** None. **L. Theresa:** None. **G. Megan:** None.

150. HPA-Axis Dysregulation, Posttraumatic Stress, and Maladaptive Grief in Parentally Bereaved Children: Individual and Environmental Correlates

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Background: Parentally bereaved children may be at a particularly high risk of developing PTSD (Melhem *et al*., 2008; Pfeffer *et al*., 2007), as well as maladaptive grief reactions (e.g., complicated grief). However, few studies have examined the biological, psychological, and environmental factors that may be associated with the development of these outcomes in this population. Evidence suggests that the hypothalamic-pituitary-adrenal (HPA) axis and the primary steroid hormone produced by this system, cortisol, play a key role in mediating the negative impact of stress on health and impacting onset, course or pathophysiology of psychiatric disorders. Although there is emerging evidence to suggest that the cortisol awakening response is negatively associated with PTSD in children (Adam & Kumari, 2009), no studies to date have examined the relation between cortisol and complicated grief in bereaved children. Psychological factors such as avoidant coping styles may be particularly relevant to both HPA axis reactivity and posttraumatic stress symptoms (PTSS) in adults (Tull, Sheu, Butler, & Cornelious, 2005). These relations have yet to be adequately examined in children, despite growing evidence that avoidant coping is prevalent among traumatized youth and has been linked with childhood PTSS (Kaplow *et al*., 2005). Highly stressful experiences in childhood activate attachment proximity seeking. The availability of an attachment figure helps to reduce fear and serves as a biobehavioral regulator (Shear *et al*., 2007). When the caregiver's availability is disrupted to a significant degree (e.g., by their own maladaptive grief), the child is likely to be at higher risk for experiencing clinically significant psychological distress (Lin *et al*., 2004), alterations of biological stress systems, and derailment from a normal developmental trajectory (Lieberman *et al*., 2003). The primary goal of this pilot study is to examine the relations between biological (HPA-axis dysregulation), psychological (avoidant coping), and environmental (parental complicated grief) factors as well as bereavement-related psychopathology (PTSS and complicated grief) among parentally bereaved children.
Methods: The study sample consisted of 26 bereaved children (11 males, 15 females) who had lost a parent within the previous six months and 23 of their surviving caregivers. Children were between the ages of 7 and 12 (M = 9.4; SD = 1.92), with 21 children identifying themselves as Caucasian, 3 African American, 1 Hispanic, and 1 Asian American. Surviving parents and children were asked to provide saliva samples in their home at three different time points (upon wake-up, 30 minutes after wake-up and

in the evening) over the course of 3 days, beginning on the day following their interview. HPA axis dysregulation in both child and parent was measured via cortisol awakening response. Paper-and-pencil measures included reliable and valid assessments of PTSS and complicated grief (in both parent and child) and avoidant coping in the child.
Results: No significant differences in child cortisol responses were identified as a function of age, gender, or race. Results showed a significant relation between a dampening of the cortisol awakening response and increased PTSS ($r(20) = -.54$, $p < .05$) as well as complicated grief symptoms ($r(20) = -.57$, $p < .01$) in parentally bereaved children. The child's use of avoidant coping was also associated ($r(20) = -.68$, $p = .001$) with a dampening of the cortisol awakening response. Interestingly, neither the surviving parent's cortisol response, nor PTSS, were associated with the child's cortisol response. However, higher levels of complicated grief in the parent was associated with a dampening of the cortisol awakening response in children ($r(26) = -.56$, $p < .01$).
Discussion: Taken together, these findings suggest that HPA-axis dysregulation may be an important biomarker for PTSS and maladaptive grief reactions in children. It also appears that individual and environmental factors are associated with a dampening of the cortisol awakening response. In particular, children who have a greater tendency to use avoidant coping strategies and children who have parents with complicated grief seem to be at greater risk for experiencing HPA-axis dysregulation. These findings have important implications for the prevention of PTSS and complicated grief reactions in parentally bereaved children. Psychosocial interventions that focus on reducing children's use of emotional suppression or avoidant coping and reducing parental complicated grief may indirectly reduce the likelihood of PTSS and complicated grief in bereaved children via the HPA-axis.
Disclosure: J. Kaplow: None. **A. Prossin:** None. **D. Shapiro:** None. **B. Wardecker:** None. **J. Abelson:** None.

151. Differential Effects of Estrogen Preparation on Changes in Regional Cerebral Metabolism in Postmenopausal Women

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Background: Estrogen has been shown to exert neuroprotective effects in a variety of in vitro and in vivo models of brain pathology which has not been clearly established in women. Previously we reported that 17-β estradiol-based (estradiol) preparations significantly differ from conjugated equine estrogen-based formulations (CEE) in cerebral metabolism. Here we extend our findings to regional metabolic and cognitive differences of estradiol versus CEEin postmenopausal women at risk for ADin a prospective randomized trial of discontinuation-or-continuation of hormone therapy (HT). Further, we assessed telomere length as a marker of stress responsivity in relation to duration of exposure and changes in regional cerebral metabolism.
Methods: Subjects were cognitively intact postmenopausal women ages 50-65 at risk for AD and receiving estrogen-containing hormone therapy (HT) for at least one year, and randomized to continue or discontinue HT (HT + and HT-, respectively) for the 2-year study. AD risk was defined as family history of AD and/or apolipoprotein allele e4-carriership(APOE4). [F-18] fluorodeoxyglucose (FDG)-PET scans were used for the determination of patterns of regional cerebral metabolism, occuring 40 minutes post FDG injection using the CTI/Siemens (Siemens Corp, Hoffman Estates, Il) HR + tomograph (63 image planes). *A priori* regions of interest included hippocampus/parahippocampal,posterior cingulate,parietotemporal and inferior lateral temporal cortex, among others. Telomere length was measured using a quantitative PCR method in peripheral blood mononuclear cells. Imaging, cognition and telomere length were assessed at baseline and 2 years after HT randomization. In analysis, HT + and HT- women were tested for potential demographic and clinical differences using t-tests and chi-square tests. Repeated-measures analysis of variance was used to test for changes in regional brain metabolism over the 2-year observation period between HT + and HT- women. Within-group t-tests were used to contrasted differences in change over time by type of estrogen.
Results: HT + and HT- women showed no significant differences with respect to age, years of education, MMSE scores, age at menopause, length of HT use, or length of reproductive life. HT + women ($n = 28$) included 16 on estradiol and 12 on CEE, while HT- women ($n = 17$) included 13 on estradiol and 4 on CEE. Among HT- women, the most significant decline occurred in precuneous/ posterior cingulate ($t = 4.77$, $p < 0.0005$). In contrast, HT + women did not show significant decline in either hemisphere of this brain region. HT + women receiving CEE, as with HT- women as a whole, demonstrated significant decline in precuneous/ posterior regions bilaterally (left: $t = 6.48$, $p < 0.0005$; right: 16,-56,26, $t = 4.71$, $p < 0.0005$), and a significant decline in the primary visual cortex ($t = 4.33$, $p = 0.001$). No such decline was found in the HT + group as a whole. Metabolic changes were corroborated by changes in verbal memory.
Discussion: Conclusion: Continued use of estradiol ameliorated metabolic decline in posterior cingulate cortex, whereas CEE was unexpectedly ineffective in this regard. These findings have major implications for a neuroprotective role of estradiol-based HT.
Disclosure: N. Rasgon: Part 1: Bayer Pharmaceuticals (PI of a multi-site study) ADA (American Diabetes Association) – research support, Part 2: n/a, Part 3: n/a, Part 4: CURRENT: Bayer Pharmaceuticals (PI of a multi-site study) ADA (American Diabetes Association) – research support PAST: Takeda Pharmaceuticals Lundbeck Pharmaceuticals Abbot Laboratories, Inc. (past grant support), Bristol-Myers Squibb Company (past speaker), Forest Laboratories (past research support, speaker), Glaxo-SmithKline (past research support), Pfizer, Inc. (past research support & past speaker), Wyeth Pharmaceuticals. (past research support, consultant)., Part 5: n/a. **H. Kenna:** none. **T. Wroolie:** none. **C. Geist:** none. **E. Epel:** Part 1: Co-founder of Telome health Inc, a company for telomere measurement.. **C. Kroenke:** none. **J. Lin:** Part 1: Co–founder of Telome Health Inc, a diagnostic company measuring telomere biology. **D. Silverman:** none.

152. Chronic Pituitary Dysfunction after Blast-related Mild Traumatic Brain Injury

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Background: Studies of civilian traumatic brain injury (TBI) from all causes have found evidence of chronic hypopituitarism, as defined by deficient production of one or more pituitary hormones measured at least one year after injury, in 25-50% of cases. Its frequency of occurrence has not been found to be related to trauma severity. The most common anterior pituitary dysfunctions reported were growth hormone deficiency (GHD) and hypogonadism. Hypopituitarism, and in particular adult GHD, is associated with non-specific behavioral symptoms that can be mistaken for PTSD, including fatigue, anxiety, depression, irritability, insomnia, sexual dysfunction, poor concentration and memory, and decreased quality of life. Despite the high frequency of hypopituitarism after civilian TBI, the prevalence of hypopituitarism after blast-related mild TBI, the signature injury of combat in Iraq and Afghanistan, has not yet been investigated. Mild TBI (mTBI) is

characterized by brief loss or alteration of consciousness. The mechanisms of injury of blast mTBI are very complex and poorly understood. Blast is propagated directly through the skull and indirectly via blood vessels, and reflections of blast waves in a closed space can redirect and magnify their effects. The pituitary is vulnerable to compression due to its confinement in the sella turcica, and the narrow pituitary stalk (2-3 mm diameter) is subject to torsional and rotational forces resulting from brain movement.
Methods: In order to determine the frequency of pituitary dysfunction after blast-related mTBI, we are measuring pituitary and target organ hormones in blood samples taken from Iraq/Afghanistan Veterans with mTBI at least one year subsequent to their last blast exposure, and from Veterans after deployment in Iraq/Afghanistan without blast exposure. Criteria for identifying abnormal circulating levels of luteinizing hormone (LH), follicle-stimulating hormone, total testosterone, prolactin, adrenocorticotropin, cortisol, thyroid-stimulating hormone, free thyroxine, growth hormone, insulin-like growth factor-I (IGF-I), oxytocin, and arginine vasopressin (AVP) were derived from determinations of normative ranges of basal morning hormone concentrations in a group of male non-Veteran control subjects. In general, hormone concentrations below the 5th percentile or above the 95th percentile were defined as abnormal. When both pituitary and target organ hormones were measured for a given axis, a specific combination of criteria defined dysfunction of that axis.
Results: Based on the normative ranges defined by hormone measurements in control subjects, 11 of 26, or 42%, of Veterans with blast mTBI were found to have abnormal hormone levels in one or more pituitary axes. Five Veterans with mTBI were found to have probable GHD, based on age-adjusted IGF-I concentrations below the 10th percentile concentration of the reference control group. Three Veterans in the mTBI group were found to have probable hypogonadism on the basis of abnormally low testosterone and LH concentrations. Six of the mTBI group were found to have abnormal levels of the posterior pituitary hormones oxytocin and/ or AVP. None of the non-blast-exposed Veterans were found to have hormone abnormalities.
Discussion: These preliminary findings suggest that the prevalence of hypopituitarism after blast-related mTBI is similar to that in other forms of TBI. Consistent with earlier studies of TBI from all causes, GH and gonadotropin deficiencies were most frequent. Posttraumatic hypopituitarism is associated with a constellation of neuropsychiatric symptoms and diminished quality of life similar to PTSD that are largely amenable to successful treatment with hormone replacement. Routine screening for pituitary dysfunction after blast mTBI shows promise for appropriately directing diagnostic and therapeutic decisions that may otherwise remain unconsidered and for markedly facilitating recovery and rehabilitation.
Disclosure: C. Wilkinson: None. **E. Peskind:** None. **E. Colasurdo:** None. **K. Pagulayan:** None. **J. Shofer:** None.

153. The Effect of Citalopram on CRF R1 and R2 Expression in the Dorsal Raphe of a Primate Model of Differential Stress Sensitivity

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Background: We have demonstrated marked differences in the neurobiology of the serotonin system between stress-sensitive (SS) and stress resilient (SR) cynomolgus macaques characterized in a model of stress-induced amenorrhea, also called Functional Hypothalamic Amenorrhea (FHA). In our model, SS, but not SR, macaques exhibit ovulatory suppression during combined mild metabolic and psychosocial stress. Even in the absence of stress, SS animals have lower baseline estrogen and progesterone levels and lower expression of pivotal serotonin-related genes compared to SR animals [1]. Subsequently, we found that administration of the SSRI, S-Citalopram (CIT), normalized serum estrogen and progesterone concentrations in SS animals. Although SSRIs elevate extracellular serotonin, CIT did not affect serotonin-related gene expression in SS animals [2]. Rather, CIT significantly decreased CRF fiber density in the raphe of SS animals. Moreover, SS monkeys treated with CIT tended to have a higher number of UCN1-positive cells in the rostral midbrain than placebo-treated SS monkeys [3]. This study continues our investigation of the connection between CRF and serotonin systems in individuals with different sensitivities to stress-induced amenorrhea with examination of CRF receptor expression in the dorsal raphe.
Methods: SR or SS cynomolgus macaques were treated with either placebo or CIT for 15 weeks at a clinically relevant dose. CRF-R1 and CRF-R2 gene expression was detected with *in situ* hybridization using monkey specific digoxigenin-labeled riboprobes. The positive pixel area and the number of CRF-R1 and CRF-R2 positive cells were quantified by stereology and image analysis. The results were compared with two-way ANOVA followed by a post-hoc pairwise comparison.
Results: There was a significant effect of treatment and a significant interaction between treatment and stress sensitivity on CRF-R2 positive pixel area ($p < 0.004$ and $p < 0.006$, respectively) and on the number of CRF-R2 positive cells ($p < 0.023$ and $p < 0.025$, respectively). That is, the stress sensitivity determined expression in the response to CIT. SS + placebo animals had slightly lower expression of CRF-R2 in the dorsal raphe nucleus compared to SR + placebo animals as reflected in both positive pixel area and positive cell number. However, CRF-R2 positive pixel area and cell number were significantly increased in the SS + CIT compared to SS + placebo (Bonferroni pixel area $p < 0.001$; cell number $p < 0.01$). CIT had no effect on CRF-R2 expression in the SR group. The average CRF-R1 positive pixel area was nearly statistically higher in the SS groups compared to the SR groups ($p < 0.08$). Also, treatment with CIT nearly statistically reduced total CRF-R1 positive cells in both SS and SR groups ($p < 0.08$).
Discussion: CRF-R2 was lower in the raphe of SS compared to SR animals. CIT administration increased CRF-R2 only in SS animals. CRF-R1 tended to decrease after CIT administration, but the small animal number precluded significance. These data suggest that the administration of CIT reduces anxiogenic components and increases anxiolytic components of the CRF system in the midbrain serotonin network, which in turn leads to improved ovarian function. Moreover, these data raise the possibility that SSRIs may be effective in the treatment of stress-induced infertility. References: [1] Bethea CL, Centeno ML, Cameron JL. 2008 Neurobiology of stress-induced reproductive dysfunction in female macaques. Mol Neurobiol 38:199-230. [2] Lima FB, Centeno ML, Costa ME, Reddy AP, Cameron JL, Bethea CL. 2009 Stress sensitive female macaques have decreased fifth Ewing variant (Fev) and serotonin-related gene expression that is not reversed by citalopram. Neuroscience 164:676-91. [3] Weissheimer KV, Herod SM, Cameron JL, Bethea CL. 2010 Interactions of corticotropin-releasing factor, urocortin and citalopram in a primate model of stress-induced amenorrhea. Neuroendocrinology 92:224-234.
Disclosure: O. Senashova: None. **C. Bethea:** None.

154. Risk for Psychosis: HPA-Axis Dysregulation and Childhood Trauma

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Background: An emerging literature suggests that experience of childhood trauma confers increased risk for developing psychotic

symptoms in adolescence and young adulthood. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been posited as a potential mechanism linking early trauma to later psychosis; however, little prospective research has examined support for this model. Individuals diagnosed at clinical high risk (CHR) for psychosis show an increased risk for imminent psychotic disorder (35% over 2.5 years), far greater than the general population or even genetic high-risk populations. CHR youth are, therefore, an excellent group to assess for risk biomarkers.
Methods: CHR participants age 12 to 30 (N = 54) and a group of age-matched healthy control adolescents (N = 33) were assessed in a prospective, longitudinal cohort design. All participants completed comprehensive clinical and neuropsychological batteries at study entry, with follow-up for CHRs at 6, 12 and 24 months. Participants in both groups provided salivary cortisol samples to assess diurnal cortisol rhythms, response to a social evaluative lab stressor task and response to administration of dexamethasone.
Results: More CHR participants reported a history of traumatic events prior to age 13 (61%) than did healthy controls (24%; z = 3.7, p < .001). At baseline, trauma history was significantly negatively correlated with GAF score (r = -.33, p < .01) and positively correlated with affective symptoms (r = 0.41, p < .01). CHR patients also showed a trend towards slower cortisol recovery after the Trier Social Stress Test compared to healthy controls (F = 2.38; p = 0.07). By 6-month follow-up, 4 of 35 UHR subjects (11%) had converted to a full psychotic disorder and 7 of 21 subjects had converted by 12 months (33%). Preliminary analyses predicting conversion from baseline clinical variables and cortisol measurements will also be reported.
Discussion: Consistent with the growing literature on stress, trauma and schizophrenia, adolescents and young adults at clinical high risk for psychosis report experiencing more traumatic events in childhood than their age-matched healthy control counterparts and may show an abnormal stress response. Ongoing work will assess whether early trauma is related to HPA axis functioning and conversion to psychotic disorder over time.
Disclosure: R. Loewy: Part 4: Genentech, Inc. **R. Pearson:** None. **B. Stuart:** None. **D. Mathalon:** Part 1: Pfizer Corporation-consulting, Part 4: AstraZeneca Pharmeceuticals. **S. Vinogradov:** Part 1: Brain Plasticity, Inc. - consultant.

155. Stress Reactivity in Persons at Familial Risk for Schizophrenia

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Background: Persons with schizophrenia have a dysregulated stress response that includes the autonomic (ANS), and endocrine (e.g. cortisol) systems. While the mechanism is not well understood, altered stress reactivity may increase schizophrenia vulnerability by influencing brain development. First degree relatives are at elevated risk for schizophrenia, and as such may express altered indices of stress reactivity. In this study we investigate the cortisol and ANS responsivity to a laboratory stressor in adolescents and young adults (ages 16-24) who are siblings of patients with schizophrenia (n = 20), their schizophrenia relations (n = 10), and healthy comparison subjects (n = 20). Preliminary results are presented for a subgroup of subjects.
Methods: We use the Montreal Imaging Stress Task (MIST) that employs computerized challenging mental arithmetic in the presence of negative social evaluative 'feedback.' Cognitive load (arithmetic difficulty) is automatically increased based on each individual's performance. Salivary cortisol is assayed at six time points during and after each stress test, collected with Salimetrics™ oral swabs and assayed using the Salimetrics™ High Sensitivity cortisol assay. Continuous ECG is measured with a standard 3-lead configuration (1000 Hz sampling rate). Customized software is used to calculate inter-beat intervals (time in ms) between R-wave peaks.Parasympathetic activity is indexed with the high spectral frequency band, HF-HRV (between 0.15 – 0.40 Hz), and the balance of sympathetic and parasympathetic activation (SNS) with the ratio of (0.04-0.15 Hz) to high frequency HRV power (LF/HF HRV).
Results: The FHR- control subjects (n = 5) show the expected robust elevation of cortisol in response to the stress task, with normal return to baseline. The FHR + (n = 10) subjects have a blunted response, similar to their siblings with schizophrenia (n = 3). Compared with FHR- controls (n = 6), FHR + subjects (n = 10) have (1) delayed recovery in HR response to MIST stress, (2) increases in sympathetic activity indexed by LF-HF HRV ratio during recovery with incomplete return to baseline levels, and (3) impaired parasympathetic withdrawal during stressor and incomplete return to baseline during recovery (HF-HRV).
Discussion: The preliminary analysis indicates that adolescents and young adults with a sibling with schizophrenia have an altered stress response as measured by salivary cortisol and heart rate variability. Blunted salivary cortisol response to a stressor is consistent with a chronically elevated cortisol and a chronic stress state. The cardiovascular results are indicative of poor regulatory control of the 'fight or flight' response to stress, including delayed parasympathetic recovery from a stressful event. The stress task used in this study is consistent with day-to-day stressors, suggesting that first degree relatives of persons with schizophrenia may showed impaired regulation of response to mild stressful events. The consequences altered cortisol and ANS system function on adolescent brain development are not well understood, and deserve further study. Cortisol and ANS system stress reactivity are modifiable in persons with medical disorders and in healthy persons under chronically stressful life situations and thus may be modifiable in this population, potentially influencing schizophrenia risk.
Disclosure: D. Perkins: Part 1: Consultant: Sunovion, Eli Lilly, Endo Pharmaceutical, Genetek, Merck Speakers Bureau: Eli Lilly, Part 2: Sunovion. **K. Grewen:** None. **A. Belger:** None. **S. Ramsey:** None. **K. Lansing:** None. **J. Evans:** None.

156. Predicting Seizure Threshold and Individualizing Seizure Therapy Dose by Pulse Amplitude Adjustment

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Background: In present practice, electroconvulsive therapy (ECT) and magnetic seizure therapy (MST) are delivered with high, fixed current amplitude set at the maximal stimulator output (800 or 900 milliamperes (mA) for ECT and 100% maximal stimulator pulse amplitude (%MA) for MST). Using a high ECT amplitude has potential drawbacks because 800-900 mA is considerably higher than the minimum necessary to induce a seizure: (a) historical studies claim that ECT current amplitudes substantially lower than 800–900 mA can induce safe and effective seizures; (b) MST induces seizures at very low induced currents compared with ECT, even when MST is given at maximal stimulator output; (c) the electric field induced in the brain by conventional ECT current amplitudes is several fold higher than the neural activation threshold, resulting in widespread direct stimulation of the brain (Deng *et al*, J Neural Eng, 2011). Using fixed ECT and MST pulse amplitude for all patients may have drawbacks because individual variation in anatomy results in variable strength of the induced

electric field and, consequently, potentially variable clinical outcomes (Peterchev *et al*, J ECT, 2010). We examined the value of pulse amplitude adjustment as a means of individualizing dosage requirements for ECT and MST. Titrating seizure threshold (ST) by manipulating pulse amplitude could provide more appropriate stimulus individualization than conventional titration by manipulation of number of stimulus pulses, because it can compensate for individual anatomical and neurophysiological variability. Pulse amplitude adjustment may also result in seizure initiation with lower-than-conventional pulse amplitudes. We hypothesized that the motor threshold (MT)—the amplitude required to elicit a muscle twitch—would predict pulse-amplitude titrated ST, since the MT captures individual variability in anatomy and neurophysiology. This could have significant clinical value, since known predictors of ST when titrated using traditional means explain only a small proportion of the ST variance.
Methods: MT and ST were titrated for MST and ECT in *macaca mulatta*. Anesthesia included ketamine 5–10 mg/kg and xylazine 0.35–0.7 mg/kg i.m., and methohexital 0.5 mg/kg and succinylcholine 3.5 mg/kg i.v. MST was delivered in 9 subjects (age = 11.9 ± 2.6 years, range = 9.3–16.3 years) with 0.36-ms cosine pulses through a 10cm diameter circular coil on vertex. ECT was delivered in 7 of these subjects (age = 12.3 ± 2.9 years, range = 10.1–16.5 years) with 0.2 ms rectangular pulses through 3.5 cm diameter electrodes in BL configuration and 2.5 cm diameter electrodes in RUL configuration. MT was defined as the minimum stimulus pulse amplitude needed to achieve a 50 mV peak-to-peak motor evoked potential in the first dorsal interosseus muscle for at least five out of ten trials. MT determination was followed by ST titration consisting of 10 s, 50 pulses per second trains with increasing amplitude. The ECT train was unidirectional with cathode (negative) on the right. MST induced in the head clockwise initial current phase. MT and ST titration was repeated 3 times for each condition in each subject.
Results: Average MT was 64 ± 18 mA for BL ECT, 79 ± 24 mA for RUL ECT, and 20 ± 3.5%MA for MST. Average ST was 176 ± 48 mA (range = 111–222 mA) for BL ECT, 160 ± 65 mA (range = 81–284 mA) for RUL ECT, and 38 ± 4.4% MA (range = 33–46% MA) for MST. The STs were significantly lower than conventional ECT and MST pulse amplitudes ($p < 0.0001$). For all three modalities ST and MT were highly correlated ($R^2 = 0.80$, $p < 0.01$). ST was not correlated with age.
Discussion: We demonstrated the feasibility of titrating ECT and MST ST by pulse amplitude adjustment, and introduced a method for rapid determination of MT with ECT. We show that seizures can be induced with stimulus amplitudes lower than conventional by 4.5–5.6 times for ECT and 2.6 times for MST. Use of lower stimulus amplitudes could improve the stimulation focality and should be explored as a means of lowering side effects. The data suggest that the ECT stimulus amplitude should not only be reduced but also individualized, since there is a wide individual spread of ST values. The MT is a very strong predictor of amplitude-titrated ST and should be evaluated as a faster and safer alternative to empirical ST titration. In this small sample, age did not predict amplitude-titrated ST. This work motivates the study of amplitude adjustment in clinical ECT and MST. ECT devices should be designed to provide an adequate range of pulse amplitude adjustment (e.g., 100–900 mA) to enable amplitude-titration studies.
Disclosure: A. Peterchev: Part 1: Dr. Peterchev is inventor on patents, patent applications, and invention disclosures on TMS/MST technology; he is inventor on a patent and patent applications on TMS/MST technology licensed to Rogue Research; he has received a research grant from Rogue Research and equipment donations from Magstim, MagVenture, and ANS/St. Jude Medical; he is also supported by NIH grant R01MH091083 and Wallace H. Coulter Foundation Translational Partners grant., Part 2: Duke University, Part 3: N/A, Part 4: Dr. Peterchev has received a research grant from Rogue Research and equipment donations from Magstim, MagVenture, and ANS/St. Jude Medical; he is also supported by NIH grant R01MH091083 and Wallace H. Coulter Foundation Translational Partners grant., Part 5: N/A. **M. Rosa:** None. **S. Lisanby:** Part 1: Honoraria for editing or writing books/chapters from: Oxford University Press, Wolters Kluwer Health, Cambridge University Press. Honoraria for giving Grand Rounds or other CME lectures (Saint Lukes Hospital, Maimonides Hospital, NYU, Lenox Hill Hospital, New South Wales, Australia). Honoraria for reviewing grants (NIH). Coinventor on a patent application for magnetic stimulation technology (not licensed, no revenues). Member of the Board of Directors of the International Society for ECT and Neurostimulation (unpaid). Research grants and equipment support listed in Part 4 below., Part 2: none, Part 3: none, Part 4: Research grants from: ANS/St. Jude Medical, Neuronetics (past), Cyberonics (past), Brainsway Equipment support from: Magstim, MagVenture, Part 5: N/A.

157. First Human Use of Focal Electrically-Administered Seizure Therapy [FEAST] Shows Feasibility, Safety, Clinical Benefits and Short Reorientation Time

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Background: Electroconvulsive therapy (ECT) remains the most effective acute antidepressant treatment for TRD, but with significant risks of cognitive side effects. The efficacy and side effects of conventional ECTare contingent on the anatomic positioning of electrodes and stimulus dosage, which determine where the electrical current flows, and where the seizure initiates and spreads. A technique that could spatially target the prefrontal cortex, and reduce the involvement of medial temporal lobe regions, may preserve the efficacy of ECT while simultaneously reducing the memory (amnestic) and other side effects.Focal Electrically Administered Seizure Therapy (FEAST), a novel electrode placement and geometry with unidirectional stimulation, has been proposed as a means to initiate seizures focally in the prefrontal cortex prior to secondary seizure generalization. Conventional ECT uses bidirectional trains and relatively high current amplitudes, which cannot be focused in this manner. Early FEAST work in a preclinical model and computer simulations showed the promise of this approach. We report here on the first human clinical application.
Methods: Seventeen depressed unmedicated adults (5 males; 3 bipolar affective disorders; age 53 ± 16; length of depressive episodes 225.7 ± 257.3 weeks) were recruited at NYSPI or MUSC after being referred to ECT in this IRB and Investigational Device Exemption US FDA approved feasibility study. Open-label unidirectional FEAST was administered with a modified Spectrum 5000Q device in a traditional ECT dosing regimen of 3 treatments per week until response. A round stimulating electrode (0.75", 1.0" or 1.25") was placed anteriorly above the center of the right eyebrow. A larger oblong posterior electrode (1 × 2.5" or 2 × 3") was placed tangential to the midline and extended across the right supplementary motor cortex (similar to D'Elia posterior electrode placement). Five parameter combinations were used at the initial titration session, with charges ranging from 5.4 to 43.2 mC. Treatments were 6 to 9 times seizure threshold ranging from 97.2 to 384.0 mC. Comprehensive clinical and cognitive assessments were performed at baseline, end of course, 2, 4 and 6 months follow-up. Time for orientation recovery, a predictor of long-term amnestic effects, was acquired on all treatments.
Results: The average number of treatment sessions per patient was 8.8, the median was 10, with a range 4-14. After the course of

FEAST, there was a 42.1 ± 38.3% improvement in Hamilton Rating Scale for Depression ($HRSD_{24}$) scores compared to baseline (32.7 ± 6.8, 17.7 ± 11.2; paired 2 tailed t-test p = 0.0004). At the end of the course, 8 of 17 patients met criteria for response (50% improvement from baseline) and 5/17 met remission criteria ($HRSD_{24}$£10). Modifications in stimulation parameters over the course of the study including sizes of electrodes resulted in improved efficacy, with only 1 of the first 9 patients achieving remission compared to 4 of the last 8. Patients achieved full re-orientation in 16.9 ± 7.5 minutes from stimulus delivery or 7.3 ± 9.6 minutes (median = 3.6) from when eyes first opened. None suffered noticeable cognitive worsening from baseline at 2 or 6 months follow up. There were 2 first-degree burns at the anterior site of stimulation with the smaller 0.75" electrode.

Discussion: In this open-label feasibility study, the FEAST technique produced clinically meaningful antidepressant improvement, and with limited cognitive side effects as indexed by a short time to reorientation. A larger frontal electrode sized at 1.25" improved dynamic impedance to within acceptable and safe ranges for electrical stimulation.Additional work is needed to refine the FEAST technique, which shows promise as a novel refinement of ECT.

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158. Relapse Rates in Psychotic Depression are Lower than in Non-psychotic Depression after a Successful Course of Electroconvulsive Therapy (ECT)

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Background: The treatment of patients with psychotic depression presents different challenges than that of patients with non-psychotic depression. We have previously reported differential response rates to an acute course of Electroconvulsive Therapy (ECT) in psychotic vs. non-psychotic depression. Patients with psychotic depression respond in higher rates and more rapidly than those with non-psychotic depression. In this report, we compare relapse rates of psychotically and non-psychotically depressed patients who responded to an acute course of ECT and were treated with either a fixed course of continuation ECT (C-ECT) or continuation pharmacotherapy (C-PHARM) for six months.

Methods: In an NIMH-funded, multicenter, randomized study conducted by the Consortium for Research on ECT (CORE) 531 patients were treated with acute ECT. ECT was administered with bilateral (bifrontotemporal) placement at 1.5 times the seizure threshold. Remission was defined as 2 consecutive ratings of the 24-item Hamilton Rating Scale for Depression (HRSD-24) of 10 or lower. 201 remitters entered a 6-month continuation phase. During that phase, patients were randomized to receive either a fixed course of 10 C-ECT without medications, or the combination of lithium and nortriptyline. No antipsychotics were used in either study arm. We stratified for psychosis status. Relapse was defined as HRSD higher than 17, or hospitalization. We compared relapse rates between psychotic and non psychotic patients.

Results: The intent to treat sample consisted of 184 patients, 66 with psychotic depression and 118 with non-psychotic depression. Demographic characteristics did not differ between the 2 groups.

The six month relapse rates were 28.8% (N = 19) among the psychotic patients and 44.9% (N = 53) among the non-psychotic (p = 0.009), while 26.3% did not complete the study or exited prematurely. Among the patients in the C-ECT group, 32.3% of the psychotic patients relapsed, compared to 44.8% of the non-psychotic patients (p = 0.159). In the C-PHARM group, the relapse rates were 25.7% and 45% respectively (p = 0.22) Among patients with psychotic depression who received C-ECT, 48.4% remained remitted and among those who received the combination of lithium and nortriptyline, 65.7% remained remitted.

Discussion: Relapse rates after a successful course of bilateral ECT are lower in psychotic depression than in non-psychotic depression in a follow-up period of six months. Relapse rates among patients with psychotic depression are lower in patients receiving the combination of lithium and nortriptyline than in patients receiving continuation ECT without medications on a fixed schedule. These data support the argument that psychotic and non-psychotic depression represent different nosologic entities and may require different treatment algorithms.

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159. Narp Mediates the Antidepressant Effect of ECT

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Background: Thirty percent of depressed patients do not adequately respond to existing pharmacologic treatment. Electroconvulsive therapy (ECT) is the most effective and has the most rapid onset of all antidepressant therapies and is indicated in medication-resistant depressed patients. Whereas patients improve with medications over several weeks, ECT recipients experience the largest improvement after the 1st treatment and over half show initial response by the 3rd ECT. However, ECT requires anesthesia and causes memory loss. Therefore, identifying key pathways and molecular mechanisms involved in ECT response could help foster development of more effective and rapidly acting antidepressant agents. Existing theories of antidepressant mechanism of action seem incapable of explaining the rapid ECT response. For example, hippocampal neurogenesis which appears to be necessary for the antidepressant effect of

pharmacologic agents could not explain the rapid ECT effect as it takes weeks for new neurons to be integrated into neuronal networks. As immediate early genes (IEGs) are rapidly and robustly induced by ECS and regulate synaptic plasticity, we hypothesized this class of genes could mediate the rapid ECT effect. We chose to test this hypothesis by focusing on Narp, an IEG induced by ECS, for several reasons: (1) Narp accumulates in hippocampus with repeated ECS, (2) Narp is enriched in neurons projecting to nucleus accumbens from key limbic regions such as medial prefrontal cortex and amygdala, (3) Glutamatergic dysregulation has been implicated in the pathophysiology of depression, and Narp regulates AMPA receptor clustering.

Methods: To evaluate the role of Narp in ECS responsiveness, we assessed both naïve and chronically stressed Narp KO and WT mice. Our chronic stress protocol included solitary housing, and a rotating regimen of daily restraint stress for up to 6 hours and wet bedding for a total of 25 days. ECS responsiveness was assessed 48 hours after 5 daily ECS or sham treatments. We monitored both behavioral measures of antidepressant response and hippocampal BDNF protein levels.

Results: Both naive and stressed WT mice treated with ECS, when compared with sham treated WT mice, showed less immobility on both forced swim and tail suspension tests, two commonly used assays of antidepressant responsiveness. Stressed WT mice treated with 5 daily ECS also showed an increase in sucrose preference compared with WT mice treated with sham stimulation. However Narp KO mice treated with ECS failed to respond on any of these behavioral measures of antidepressant efficacy. We also found Narp KO mice do not differ from WT controls in locomotor activity after 5 daily ECS when monitored in an activity chamber, indicating their lack of responsiveness on antidepressant behavioral measures is not due to lower activity levels post-ECS. Moreover, the seizures themselves appear normal in Narp KO mice as indicated by seizure duration and pattern of c-fos activation. Analysis of BrdU labeling in the dentate at 60 hours following the last ECS indicated hippocampal neurogenesis triggered by ECS is likewise unaffected by Narp deletion.As BDNF is thought to mediate antidepressant behavioral responses induced by chemical agents, we also monitored BDNF protein levels in the hippocampus by ELISA. Whereas 5 daily ECS administered to naïve or chronically stressed WT mice increases BDNF in the hippocampus, ECS fails to increase BDNF in Narp KO mice.

Discussion: Our findings indicate Narp plays a key role in mediating the antidepressant effect of ECS and suggest it may do so by mediating the rise in hippocampal BDNF induced by this treatment. In contrast, ECS episodes themselves appear to be unaffected as induction of c-fos and the rise in BrdU labeling in dentate gyrus are unaffected in Narp KO mice. Thus, we infer Narp deletion blocks the ability of seizure activity to elevate BDNF levels and induce behavioral changes indicative of antidepressant response. While it is tempting to conclude these are mechanistically related, further studies are needed to test this hypothesis and to elucidate the mechanisms by which Narp regulates BDNF expression and signaling. In ongoing studies we are utilizing viral vectors to determine which of several candidate anatomical pathways that express Narp mediate the impaired behavioral and BDNF responses to ECS. We are also checking whether Narp deletion affects response to antidepressant medications. If so, that would indicate Narp has a generalized effect on antidepressant efficacy. If not, that would indicate Narp has antidepressant properties selective for ECS which could potentially be harnessed to develop novel pharmaceuticals.

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160. Effects of Omega-3 Fatty Acid Supplements on PET Quantification of Regional Brain Glucose Metabolism in Depressed and Healthy Volunteers

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Background: We have previously shown in major depression that plasma glycerophospholipid levels of the omega-3 polyunsaturated fatty acid (PUFA), docosahexaenoic acid (DHA), correlated with regional metabolic rates of glucose (rCMRglu) obtained through [^{18}F]fluorodeoxyglucose positron emission tomography (FDG-PET). We now extend these findings with a prospective study of effects of 6 weeks of omega-3 PUFA supplementation on rCMRglu in depressed and healthy volunteers.

Methods: Participants with DSM IV SCID-diagnosed major depressive disorder (N = 14) and healthy volunteers (N = 7) provided informed consent for this study, which was approved by the IRB. Depressed participants were free of psychotropic medications except for one person on mirtazepine. FDG-PET and MRI scanning were performed before and after all participants received 4 g/d of fish oil supplementation (1.6 g/d of eicosapentaenoic acid [EPA] and 0.8 g/d of DHA). FDG-PET data was reconstructed into images, after which motion correction, coregistration to MRI, and generation of regional time activity curves were performed using a single venous sample taken at 40 minutes. Data were analyzed quantitatively using our validated method, the simultaneous estimation of input function (SIME). Regions of interest (ROIs) were determined using a multiple atlas, multi-label automated probabilistic approach. Clinical response was defined as 50% or greater improvement on the17-item Hamilton Depression Rating Scale (HAM-17). The plasma glycerophospholipid fatty acids DHA, EPA, and arachidonic acid (AA) were quantified using a new, high-throughput method of Glaser *et al* (2010) and expressed as a percentage of total PUFA (DHA%, EPA%, AA%). Independent t-tests were used to compare depressed and control groups with regard to clinical characteristics, and to compare fish oil responders and non-responders with regard to PUFA% levels. Pre and post-treatment plasma levels of PUFA% and HAM-17 scores were compared using paired t-tests. Linear regression was used with individual PUFA% values as independent variables and regional rCMRglu values as dependent variables. Statistical analyses were performed using SPSS (PASW, Release 18) for Mac. No corrections were made for multiple comparisons.

Results: There were no differences between patients and controls in terms of sex, age, PUFA levels pre- or post-supplementation, or the percent change in any PUFA after supplementation. All PUFA% levels changed highly significantly after supplementation

(mean % change in the entire sample for DHA%, 29.2% ± 23.8, t = 5.65, df = 20, p < 0.0001; EPA%, 62.8% ± 26.9, t = 5.33, df = 20, p < 0.0001; AA%, -14.5% ± 18.5, t = −3.16, df = 20, p = 0.005). Within the depressed group, HAM-17 scores were significantly improved after fish oil treatment (baseline mean score = 16.8; post-treatment mean score = 11.6; t = 2.78, df = 13, p = 0.015); 5 out of 14 were full responders (baseline mean score = 17.4; post-treatment mean score = 4.8; t = 9.78, df = 4, p = 0.001). Initial PUFA levels did not predict clinical response. Post-supplementation levels of plasma DHA% (t = 2.17, df = 12, p = 0.05), but not EPA% (t = −0.50, df = 12, p = 0.63) or AA% (t = 0.51, df = 12, p = 0.61), distinguished between responders and non-responders. Neither baseline nor post-supplementation levels of PUFAs correlated wtih rCMRglu. Severity of depression after supplementation also did not correlate with FDG regional uptake or with change in uptake from pre to post-supplementation. Voxel-based analyses are in progress.

Discussion: We have previously reported a cross-sectional correlation of PUFAs with PET regional FDG uptake in specific brain regions using voxel-based analyses. In this prospective study of 6-week fish oil supplementation in major depression, we found that increased plasma DHA% was associated with clinical improvement but did not increase brain FDG uptake in ROI analyses. Supported by MH079033 awarded to Dr Sublette.

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161. Inflammatory Cytokines Are Associated With Exercise Augmentation Treatment Outcome For Major Depressive Disorder

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Background: Depression is associated with peripheral and central inflammation, and antidepressant treatment is known to correlate with a reduction in inflammation. Exercise has been shown to be an effective treatment for depression and to be anti-inflammatory, but it is not known if MDD related inflammatory markers show a reduction with exercise or if these changes correlate with depression outcomes.

Methods: In the Treatment with Exercise Augmentation for Depression (TREAD) study (Trivedi *et al*, J Clin Psych 2011 May;72(5):677-84), subjects with MDD who were partial or non-responders to SSRIs participated in a structured, dosed, 12-week exercise treatment regimen. One hundred five subjects provided a baseline blood sample, and 73 subjects provided an additional week 12 sample. Samples were analyzed for Interleukin 1-beta (IL-1â), Tumor Necrosis Factor-alpha (TNF-á), Interferon-gamma (INF-ã), and Interleukin 6 (IL-6) using ELISA. Spearman's correlations were used to determine the relationship between change in cytokine levels and symptomatic outcome. MANOVA was used to determine whether the dose of exercise affected cytokines as a set and a similar ANOVA examined the relationship between exercise dose and each cytokine individually. Mixed effects modeling was used to determine the effect of each baseline cytokine level on outcome.

Results: There was no change in any cytokine over the 12 week period in the whole sample or in either dose group. There was also no significant relationship between dose of exercise and the level of each cytokine as a group or individually. Change in IL1-â and change in $HRSD_{17}$ (p = 0.0455), IDS-C (p = 0.0441) and IDS-SR (p = 0.0470) scores were each inversely related, correlations were also significant for the high dose group for the HRSD (p = 0.0366) and IDS-SR (p = 0.0474). Baseline TNF-á level was associated with a slower rate of change on the IDS-C (p = 0.0024).

Discussion: Our results support prior findings that inflammation plays a role in treatment outcomes for MDD. In particular, we replicated for the first time in an exercise sample, two findings from prior studies of SSRI treatment – that higher TNF-á at baseline may be associated with poor outcome, and that IL-1â decrease correlates with symptom improvement. These results reemphasize the importance of inflammation in MDD, and support continued research into the mechanisms of exercise treatment.

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162. The Combination Of rAAV/GDNF With Fetal Ventral Mesencephalic Transplants in Parkinsonian Monkeys does not Significantly Improve Behavioral Outcome over Either Treatment Alone

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Background: Intracerebral injections of viral vectors harboring glial-derived neurotrophic factor (GDNF) to the dopamine-depleted nigrostriatal system has been reported to be beneficial in animal models of Parkinson's disease. Grafting of fetal dopamine precursor cells from the ventral mesencephalon (VM) to this region has also been reported to be beneficial. GDNF delivery has been reported to augment the growth and distribution of VM tissue implants, and these strategies have led to clinical trials in Parkinson's patients. Here, we combined these two to determine if the benefits of increased cell survival and increased outgrowth would improve the anti-Parkinson effects in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated monkeys, one of the best models for predicting effects in Parkinson's patients.

Methods: 27 adult male St. Kitts green monkeys (*Chlorocebus sabaeus),* following MPTP treatment, were placed into four treatment groups. Subjects in all groups received stereotaxic injections bilaterally, in the left and right posterior caudate and putamen. In the fetal tissue (VM) group, subjects received 15 µL of

saline and tiny pieces of tissue from the ventral mesencephalon of embryonic 42 to 48 day fetuses delivered by hysterotomy. In the rAAV/GDNF vector group, subjects received 20 μL of a rAAV5-hu-GDNF vector. In the fetal tissue with GDNF vector group (VM/GDNF), subjects received 20 μL of rAAV5-hu-GDNF vector and VM fetal tissue. In the sham group, subjects received 15 μL phosphate buffered saline. Subjects in each group were balanced according to severity of parkinsonian symptoms. Four observers with inter-rater reliability greater than 95% (Kendall's coefficient of concordance) scored the monkeys before and after MPTP treatment and for 8 months post surgical injections. Monkeys showing moderate to severe parkinsonism were analyzed behaviorally using our published methods for assessing parkinsonism and other natural behaviors. At the end of the experiment, samples were taken from standard locations in the nigrostriatal system and frozen in liquid nitrogen for determination of dopamine and homovanillic acid and GDNF concentrations. Vector effects were also evaluated histologically.

Results: The mean Parkinson's Score in all groups analyzed behaviorally after MPTP treatment was a severe level comparable to a Hoehn & Yahr score of 5. Repeated measures analysis of variance, with post hoc tests using Student Newman Keuls at $p < 0.05$, identified significant differences in recovery of the various groups; the sham treated animals did not improve behaviorally, whereas all three experimental groups showed significant improvements (with significant group × time interactions). Although there was no significant behavioral difference between treatment groups, there was a difference in dopamine-related measures: post-mortem tissue analysis revealed elevated striatal dopamine levels in all experimental groups, and a significantly higher level in the VM/GDNF vector treatment group compared to VM or rAAV/GDNF groups. Striatal GDNF levels in monkeys that received rAAV5/GDNF reached 20-50 ng/mg protein in the vicinity of the injection site, while without GDNF overexpression the level in the striatum was 0.2-0.3 ng/mg protein.

Discussion: All three treatments showed improvement compared with sham animals. However, the combination of fetal precursors with vector-delivered GDNF did not translate into significant functional effects in the present experiment, despite there being a clear increase in striatal dopamine concentrations in the combined VM tissue plus GDNF group. It is possible that the greater increases in striatal dopamine in the VM/GDNF group did not translate to greater functional improvement because the zone of influence of the elevated dopamine did not extend significantly beyond that achieved with each strategy alone. With the potential of fetal precursors to innervate an increasing volume of striatum over time, longer outcome periods might have been necessary to demonstrate group differences, as noted in some other studies in primates, or larger study groups might be necessary. There was a consistent trend throughout the treatment period for the VM/GDNF group to rank the most improved of the four treatments. It is possible that higher levels of GDNF might have also led to some functional inhibition of dopamine release that diminished the functional effects. (Supported by USPHS U01 NS046028 & PO1 NS044281.)

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163. Normobaric Hyperoxia Treatment of Schizophrenia

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Background: Several studies of normobaric hyperoxia in neurological conditions have found positive results. The impaired energy metabolism due to mitochondrial dysfunction and frontal lobe hypofunction in schizophrenia might be improved by increasing O_2 supply to the brain. Normobaric hyperoxia may be a potential treatment for schizophrenia.

Methods: Participants in this study, outpatients suffering from chronic schizophrenia and inhabitants of community-based psychiatric institutions (hostels), underwent baseline psychiatric/cognitive assessment and were randomly assigned to either a treatment intervention of oxygen enriched air inhalation(normobaric hyperoxia of 40% FiO_2), or to regularair inhalation (21% FiO_2), through a nasal tube, for one month. Patients were given the air/oxygen inhalations during the night (mainly while sleeping), for at least 7 hours a night. After completing one month of treatment, patients were switched (crossed-over) to the other treatment intervention.

Results: Fifteen patients completed the entire study. Five additional patients completed Phase A only. There was significant improvement in total PANSS score of patients that received oxygen compared with control group. There were positive effects of oxygen on memory and attention inneuropsychological performance tests.

Discussion: The effect size is small despite the statistical significance but the patient group was extremely chronic and severely impaired. These results are a proof of concept and normobaric hyperoxia should be studied in patients with milder forms of the illness and earlier in the course of illness.

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164. Effect of Nutrients on Intrinsic Brain Activity in Lean and Obese Women

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Background: Previous investigations of the impact of food intake on brain processes have typically employed task functional magnetic resonance imaging (fMRI) in which the brain's response to visual food cues in the context of different physiological states are compared. In contrast, examination of intrinsic fluctuations in the blood oxygen-level dependent (OLD) signal provides an opportunity to study neurobiological features associated with the physiological state itself without the use of external cues. Also, the most popular designs compare ingestion of a meal to fasting or compare ingestion of glucose to water. These conditions differ in terms of taste receptor activation as well as vagal stimulation due to the absorption of nutrients within the gut. The current study employed a unique design that better isolates the effect of nutrient absorption on intrinsic brain activity. The aim of the present study was to determine if nutrient

ingestion can alter temporal dynamics of intrinsic brain activity, and if this effect differs between lean and obese women. We hypothesized brainstem regions receiving vagal input from the gut would demonstrate increased frequency of intrinsic OLDfluctuations during nutrient ingestion, consistent with vagal afferent activation.
Methods: In a two-day double-blind crossover design, 11 lean (MI19-25 kg/m^2) and 11 obese (MI30-37 kg/m^2) healthy women underwent fMRI scanning (1.5T) following ingestion of two beverages of different caloric content, but similar sweetness. Ten minutes after beverage consumption (300 kcal or 10 kcal carbohydrate drink), subjects were scanned while resting with eyes closed. The OLDsignal was subdivided into low (0.01-0.05 Hz), mid (0.05-0.12 Hz), and high (0.012-0.20 Hz) frequency bands. Relative power within each of the three frequency bands was computed for each voxel in the brain and submitted to random-effects analyses.
Results: OLDfluctuations demonstrated a shift towards higher frequencies following nutrient ingestion in nucleus tractus solitarius (NTS) for both lean and obese women. For lean but not obese women, greater NTS high frequency power was associated with decreased hunger ratings. For obese but not lean women, greater NTS high frequency power was associated with greater functional connectivity of NTS with reward and motivation-related regions (putamen and mediodorsal thalamus). In addition, group differences in temporal dynamics were demonstrated during the high calorie condition. Specifically, lean compared to obese women demonstrated greater high frequency power in dorsal anterior cingulate cortex and Brodmann's area 43, regions known to respond to gustatory input.
Discussion: This is the first study to demonstrate an impact of vagal stimulation following carbohydrate ingestion on intrinsic brain activity. Ingestion of glucose, compared to a low caloric drink of similar sweetness, was associated with differences in the oscillatory dynamics in the area of the NTS, a brainstem region that is the main target of vagal afferents from the gut and forms the main neural conduit through which visceral afferent input affects homeostasis. Frequency-based analysis of intrinsic activity appears to be a sensitive, simple measure of the brain's response to nutrient ingestion, and may be useful as a biomarker in the study of obesity. The results show a reduced relationship in obese subjects between engagement of interoceptive mechanisms (as indexed by NTS high frequency activity) and subjective hunger ratings, as well as a greater relationship between NTS high frequency activity and activity in dorsal striatum and mediodorsal thalamus. The results thus provide additional support for altered interaction between homeostatic and reward networks in obese individuals.
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165. Objective Assessment of Social Disinhibition using the Human Behavioral Pattern Monitor (hBPM) in Bipolar Disorder and Schizophrenia Patients

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Background: Disinhibition, or the reduced capacity to regulate an impulsive response to a situation, is a broad and culturally defined construct. It is typically measured indirectly using cognitive tests that assess related concepts such as premature and perseverative responding. Disinhibited behavior is a central feature among neuropsychiatric disorders such as Bipolar Disorder (BD), where manic episodes are characterized by increased risky behaviors and violation of social conduct. Yet, there has been little effort to study social disinhibition (SD) in a controlled laboratory setting. In this work, we introduce a method for objectively measuring SD, using our recently developed human open field paradigm, referred to as the human Behavioral Pattern Monitor (hBPM). This paradigm has been useful in characterizing multiple aspects of behavior in a novel environment (e.g., motor activity, spatial patterns of the activity, and object interactions), and in defining signature patterns of neuropsychiatric conditions, then validated in rodent studies of pharmacological and genetic models. To assess SD we a priori selected behaviors reflecting a lack of social conformity.
Methods: The participants were 27 healthy comparison subjects, 41 subjects with BD, and 23 subjects with schizophrenia (SCZ). All subjects were tested in the hBPM, which is an office furnished with a desk, two bookcases, two filing cabinets, two corkboards mounted on the walls, and vertical blinds covering the window. Chairs are excluded from the room to encourage exploration. Eleven engaging toys (chosen using the criteria that they are safe, colorful, tactile, can be manipulated, and likely to invite human exploration) are placed around the room, and include wearable objects (i.e., a feather mask or a pair of sunglasses), and private desk drawers. Participants were directed into the hBPM and asked to wait there while the experimenter set up other parts of the study. They were left for 15 minutes, but were not told in advance how long their wait would be, and no other instructions were given. Their activity was monitored by a digital video camera embedded in a ceiling vent. Earlier that day, each subject had signed an informed consent that they were to be videotaped. In the absence of an existing scale to objectively assess SD, we scored the video recordings for occurrence of behaviors that were deemed to violate social norms (i.e., wearing items of clothing left in the office, lying on the floor or desk, and opening someone else's desk drawer). The behaviors were compared between groups as well as to performance on a battery of tests including the Wisconsin Card Sorting Test (WCST). The WCST provides a measure of perseveration, defined as the inability to discontinue a responsethat is no longer applicable to a situation.
Results: We found that SD behaviors were significantly more likely in the BD group compared to comparison subjects (opening desk drawers, $p < .01$, wearing objects, $p < .05$ and lying down, $p < .025$), but had similar occurrence rates in the SCZ group and comparison group. In particular, the behavior of wearing an object occurred with a prevalence rate of 14.6% (6/41) in the BD group, but never occurred among the SCZ or comparison subjects. There was no significant difference in the WCST number of perseverative responses between individuals who did or did not display disregard for social norms ($F(1,59) = 0.64$, ns).
Discussion: We have previously reported that BD patients can be distinguished from SCZ subjects based upon greater interaction with novel objects. The results of this study suggest that the hBPM can also provide an objective assessment of social disinhibition within a controlled laboratory setting. Using this methodology, we have identified samples of socially disinhibited behaviors that occur among BD subjects but almost never occur in SCZ or normal subjects. Furthermore, the results suggest that violation of social norms and perseveration may be independent representations of a failure of inhibition. SD, which is a core symptom of mania, has to date been largely assessed using rating scales and self-report measures, where validity is contingent upon the subject offering accurate self-report and/or identification of these occurrences. By providing a quantifiable means to measure SD based on objective and observable behaviors, the hBPM provides a framework to better understand this core feature of mania. In particular, the hBPM enables the development of translational paradigms (i.e.,

paradigms applicable to both human and animal models), and yields opportunities to identify critical phenotypes that can serve as targets for testing novel antimanic agents.

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166. Further Evidence for the Reliability and Validity of the Massachusetts General Hospital Cognitive and Physical Functioning Questionnaire (CPFQ)

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Background: The Massachusetts General Hospital Cognitive and Physical Functioning Questionnaire (CPFQ) was developed to assess clinically relevant cognitive and physical symptoms associated with depression (M Fava, J Clin Psych 2006;67:1754-9). It consists of 7 questions pertaining to 1) motivation/interest/enthusiasm, 2) wakefulness/alertness, 3) energy, 4) ability to focus/sustain attention, 5) ability to remember/recall information, 6) ability to find words, and 7) sharpness/mental acuity, each graded on a 6-point scale. Reliability and validation work previously completed with the instrument primarily used clinical samples from outpatient settings. The purpose of the present study was to provide additional evidence for the validity and reliability of the CPFQ in a larger sample from controlled clinical trials with well-defined patient populations.

Methods: Data from 4 studies were used in the analyses. Study 1 (N = 495) was a placebo-controlled study of LY2216684 (experimental therapeutic) monotherapy for patients who met criteria for major depressive disorder (MDD). Study 2 (N = 227) was a placebo-controlled study of LY2216684 as an adjunctive treatment for patients with MDD who were partial responders to selective serotonin reuptake inhibitor (SSRI) treatment. Study 3 (N = 483) compared switching to duloxetine or escitalopram in patients who previously responded to treatment with an SSRI for MDD, but had residual apathy in the absence of depressed mood. Study 4 comprised two identical studies (analyses pooled, N = 776) conducted under one protocol to compare the efficacy and safety of duloxetine to placebo in patients with MDD. Measures included the CPFQ,HAMD-17, MADRS, Quick Inventory of Depressive Symptomatology (QIDS-SR), Fatigue Associated with Depression (FAsD) scale, and Sheehan Disability Scale (SDS). Validity was assessed by correlation coefficients and linear regression, and reliability was assessed as internal consistency via principal component analysis with Varimax rotation and Cronbach's alpha.

Results: Validity (convergent and divergent): We assessed whether patients in remission at endpoint had greater improvement from baseline to endpoint (LOCF) in the CPFQ total score than non-remitters. Remission was defined as a MADRS total score 10 (Studies 1, 2, and 3) or a HAMD-17 total score 7 (Study 4). The CPFQ total change scores for remitters vs. non-remitters, respectively, were -11.5 vs. -4.9 (Study 1, LY2216684 arm), -8.6 vs. -1.2 (Study 2, LY2216684 arm); -8.1 vs. -2.0 (Study 3, escitalopram arm); -8.1 vs. -2.1 (Study 3, duloxetine arm); and -12.0 vs. -4.7 (Study 4, duloxetine arm), all p-values $<.001$. We calculated correlations of the CPFQ measured at baseline with scales that measure depression symptoms other than mood (convergent validity) and standard measures of depressive symptomatology (divergent validity). All reported correlations were statistically significant ($p<.05$). For Study 1, for example, the CPFQ total score was more highly correlated with the FAsD summary score (0.64), FAsD impact of fatigue score (0.62), FAsD experience of fatigue score (0.56), and SDS global functioning impairment score (0.65) compared with the QIDS-SR (0.54), MADRS (0.34), and HAMD-17 total scores (0.24). A similar pattern was noted in Studies 2, 3, and 4, though not all of the studies employed the same scales. Reliability (Internal Consistency): Principal component analysis of all studies identified a strong first factor and a possible weak second factor. After rotation retaining 2 factors, CPFQ Items 1, 2, and 3 loaded more heavily on Factor 1 (e.g., Study 1: 0.70, 0.69, and 0.76, respectively) and Items 5, 6, and 7 loaded more heavily on Factor 2 (0.68, 0.73, and 0.69, respectively). Item 4 loaded more evenly on both factors (0.62 vs. 0.45). This pattern was repeated for Studies 2, 3, and 4. Cronbach's alpha indicated high internal consistency for all studies: Study 1 = 0.89, Study 2 = 0.91, Study 3 = 0.90, and Study 4 = 0.89.

Discussion: Our analyses support the validity and reliability of the CPFQ as demonstrated by the psychometrics of internal consistency, factor analysis, and convergent/divergent associations with related constructs. Although there is psychometric evidence from Cronbach's alpha that the CPFQ is unifactorial, with a meaningful and reliable total score, there may also be clinical utility in computing physical and cognitive symptom sub-scores. In conclusion, our results support the use of the CPFQ as a valuable instrument for the detection of clinically relevant symptoms, not captured by typical measures of depression, for the assessment of treatment outcomes and remission.

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167. In Vivo Monitoring of Sodium-Potassium ATPase in Euthymic Lithium-Treated Bipolar Disorder Subjects

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Background: Lithium is one of the preferred treatments for the management of Bipolar Disorder (BPD). Although this drug has shown to be remarkably effective for mood regulation, its sometimes life-threatening side effects require the use of an invasive dose-escalation procedure in order to establish a serum lithium concentration (SLC) in the range of 0.5-1.0mM. This procedure, however, is of limited success and means to directly or indirectly monitor brain lithium concentration (BLC) are, therefore, needed. Lithium Magnetic Resonance spectroscopy (Li MRS) of the whole brain has been demonstrated as a means for non-invasive BLC measurement at clinical field strengths (<3T) [1–3]. The use of a whole brain average, however, is limited by its concomitantly high partial-voluming effects and lack of spatial information. Because there is evidence that lithium competes with sodium trans-membrane channels and that lithium treatment leads to regulation of otherwise abnormal intracellular sodium concentration [4,5], normalization of brain sodium concentration (BSC) might be a surrogate marker for lihitum's effects in the brain. We demonstrate a non-invasive, Ultra-High-Field (UHF) magnetic resonance imaging protocol for concurrent mapping of BLC and BSC in BPD subjects. Our results suggest that BSC appears to be moderately elevated in the basal ganglia of lithium-treated euthymic BPD subjects.

Methods: Bipolar Subjects ($n=5$) and normal controls ($n=4$) were scanned on a whole body Magnetom TIM 7 Tesla scanner (Siemens AG, Erlangen, Germany) using an approved Institutional Review Board (IRB) protocol. During each session, standard Gradient recalled (GRE) and high-resolution T1 (MPGRAGE) proton images were acquired for spatial normalization and volumetric measurements, respectively. Lithium MRI was performed using a single-tuned, 8-channel radio frequency (RF) coil (Stark Contrast, Erlangen, Germany) and sodium MRI was carried out using a single-tuned, home-built, 16-channel RF coil. The sodium images were acquired (TE/TR = 0.3/100ms, Voxel = 0.008cc, 12 minutes) using a twisted projection imaging (TPI) acquisition[6] while lithium images (TE/TR = 0.8/1000ms, Voxel = 3.75cc, 32 minutes) were obtained using an acquisition-weighted stack of spirals sequence (AWSOS)[7]. Lithium multi-channel images were obtained as the sum of squares with no coil uniformity corrections. Sodium multi-channel images, on the other hand, were reconstructed using a sensitivity encoding (SENSE) algorithm in order to fully remove the spatial modulation due to the receiver coil profiles. After image reconstruction, the sodium images were spatially normalized using an affine transformation and the resulting spatial averages compared on a pixel by pixel basis for the two groups.

Results: Our results demonstrate that the concentration of lithium in the cerebrospinal fluid (CSF) spaces and the scalp is very high. This high accumulation is not due to the effects of RF inhomogeneity. The spatially normalized map of the sodium content in the brain of BPD subjects appears to be moderately elevated (20%) in the basal ganglia when compared to that of normal controls.

Discussion: Previous studies have provided evidence that sodium-potassium APTase activity is lower in the bipolar brain. This lower pump activity leads to an increase in the intracellular concentration and a concomitant increase in BSC. This increase in BSC appears to be supported by this study. Full characterization of this increase will require a further analysis of potential differences between the volumes of gray/white matter in the basal ganglia as such differences could lead to compartmental changes for the sodium ion and a concomitant increase in BSC without a change in pump activity. Overall, however, our results to date suggest that the measurement of sodium concentration in the brain of BPD subjects could provide critical information about the metabolic and/or morphological changes taking place during the course of the disease.

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168. Development of Novel Integrative Multistate Measures of Efficacy, Tolerability and Functional Status in Maintenance Clinical Trials for Bipolar Disorder

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Background: In clinical trials of chronic, recurrent diseases which require indefinite treatment, the proportion of time with no or minimal symptomatology as well as the tolerability and adverse health consequences of the interventions are of interest to practitioners and patients. Bipolar disorder (BD) is particularly challenging in obtaining such information. All bipolar maintenance studies have employed Kaplan-Meier (KM) survival analytic techniques, which although providing a single efficacy result, have limited generalizability, overestimate drug placebo differences, do not comport with the common illness patterns of bipolar disorder, have rates of missingness and are unable to incorporate tolerability or functionality into the efficacy measure. We report the development of a multi-state statistical technique and its performance in re-analyses of the two registration studies of lamotrigine, lithium and placebo in maintenance treatment of BD.
Methods: We sought to develop a methodological approach that would accommodate the multiple clinical states which bipolar patients experience, provide for the estimation of the duration of health states that can be entered multiple times without requiring progressive illness states and allow incorporation of utility weightings for adverse effects and estimated or individual patient valued functional status. We utilized published criteria and the results of preliminary descriptive analyses of distributions of depression and manic scores captured by MADRS and YMRS analyses to establish thresholds for the several clinical states. This tentatively named **F**unction **A**djusted **C**linical **S**tates in **B**ipolar **D**isorder Facs-BD technique (**FACS-BD**) takes a conceptually different approach to early discontinuation and "censoring", allowing tolerability and efficacy to be combined in a more general way than the non-parametric models applied in cancer trials. Statistical significance is obtained from bootstrapping estimates of the variance for the estimated times spent in each clinical state. The procedure permits sensitivity analyses of the relative effectiveness of drugs, using utility coefficients of side effects and functionality in relationship to each clinical state, thus makes explicit the degree to which the overall benefit from a treatment is affected by adverse effects and functional capability. We applied a midpoint conversion from one clinical state to another.
Results: The primary endpoint in the published registration analyses for lamotrigine and the active comparator lithium was time to intervention for a mood episode, or for a depressive or manic episode. We analyzed operationally defined manic remission, subsyndromal manic and manic syndromal states, mixed syndromal and subsyndromal states, depressive remission, subsyndromal and syndromal states for the three randomized groups. These analyses provide both parametric tabular data and multistate block figure data, yielding both clinically intuitive results as well as statistical significance and quantitative results. The multistate approach, combined with the integrated analyses modified both for degree of adverse effects and functionality, both support and differ from the results of the initial KM analyses. By FICS-BD analyses, lamotrigine is superior to placebo on some measures of depression. but benefits substantially due to its outstanding tolerability, providing only partial benefits for depressive symptomatology. In turn, lithium is superior to placebo on time spent in remission of mania, but, when the integrative analysis combining tolerability is applied, loses a major part of its advantage.
Discussion: The FACS-BD method offers advantages both for statistical and clinical generalizability over traditional LIFETEST analysis. For example, FACS-BD constrains any imputed value at dropout that extends total time beyond the time span established for the planned analyses by making the total time equal to the maximum days planned for analysis. This important step is not achievable within a KM analysis. One limitation of the FACS-BD is that if the longest time in any clinical state is censored (dropout with actual duration unknown), it is treated as uncensored. This results in an underestimate of total duration (which is known to be longer than the time of censoring but how much longer is not known), but still results in less distortion than KM procedures for such cases. FACS-BD appears to be more robust and informative of the actual patient experience in maintenance studies, and may provide scientific advantages in new drug development and registration analyses.
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169. Clinical Staging for Bipolar Disorder: Defining Empirically Derived Distinct Prognostic Patient Groups

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Background: Bipolar Disorder (BD) is characterised by significant variability in clinical trajectories and functional outcomes. There is an extensive literature on the relationship between functional outcome and clinical and cognitive predictor variables. However a key unanswered question is whether individual patients can be categorised into subgroups with distinct profiles in terms of functional outcomes. The aim of the study was to characterise prognosticsubgroups of BD patients and to define the clinical and cognitive predictors of group membership.
Methods: Latent class analysis (LCA) was applied to clinical, cognitive and functional outcome measures in a sample of 106 remitted adult BD patients.
Results: We identified two classes of patients representing a "good" (n = 50; 47.6%) and "poor" (n = 56; 52.4%) outcome group. Amongst the clinical and cognitive variables tested episode frequency and level of residual depressive symptoms, IQ and inhibitory control emerged as the most significant predictors of group membership at the $p < 0.05$ level. Their odds ratio (OR) and confidence interval (CI) with reference to the "good" outcome group were: Episode Density (OR = 4.622, CI 1.592-13.418), level of residual depressive symptoms (OR = 1.543, CI 1.210-1.969), IQ (OR = 0.969; CI 0.945-0.995), and inhibitory control (OR = 0.771, CI 0.656-0.907). Age, age of onset and duration of illness were comparable between prognostic groups.
Discussion: Our findings demonstrate that it is possible to empirically define distinct prognostic subgroups of BD patients.

Such classification requires at least two underlying dimensions one for illness severity and another for cognitive function. Future studies are required to extend and refine such classification in order to derive clinically meaningful definitions of disease staging.
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170. Prevalence and Importance of Subsyndromal Manic Symptoms during Bipolar Major Depressive Episodes

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Background: There is increasing evidence that subsyndromal manic symptoms occur frequently during bipolar major depressive episodes (MDEs) and may be a subtle form of 'depressive mixed state.' This study examines the prevalence and clinical characteristics of MDEs with subsyndromal manic symptoms at intake and during long-term follow up.
Methods: Bipolar (type I or II) patients who entered the NIMH Collaborative Depression Study (CDS) with an MDE at intake ($N = 142$) and were followed up systematically for up to 27 years (median = 20 years) were compared based on the presence or absence of concurrent subsyndromal manic symptoms. Groups were further subdivided by the presence of symptoms of overt irritability and/or psychomotor agitation, because of the high prevalence of these two symptoms.
Results: In the interest of brevity, results contained in three detailed tables in the manuscript under review are summarized here. Subsyndromal manic symptoms during bipolar MDEs were highly prevalent (73%), and were associated with significantly increased severity of depression/dysphoria in the intake episode, ($P = 0.004$) and more suicidal ideation ($P = 0.006$) and behavior (past, current, and during long-term follow-up) ($P = 0.032$; $P = 0.003$; $P = 0.029$). Median length of the intake MDE was twice as long if accompanied by subsyndromal manic symptoms (28 vs. 14 weeks). All MDEs that cycled to mania/hypomania in the intake episode had subsyndromal manic symptom(s) at intake; no purely depressive MDEs changed polarity within the episode. Overt anger/irritability and psychomotor agitation were the most prevalent subsyndromal manic symptoms (occurring in 57% and 39% of MDEs, respectively), with 24% of bipolar MDEs having both of these symptoms. Secondary analyses showed that these two symptoms accounted for most of the negative effects associated with subsyndromal manic symptoms. Compared to the group with no concurrent manic symptoms, the angry/irritable quality of MDEs (in the absence of psychomotor agitation) was associated with a significantly higher rate of future serious suicide attempts and impaired relationship with spouse/partner, as well as a longer duration of the intake episode. Psychomotor agitation (without overt anger/irritability) was associated with significantly increased depressive severity, suicidal ideation, and prevalence of suicide attempts during the intake episode and during long-term follow-up. The combination of overt anger/irritability and psychomotor agitation during bipolar MDEs was associated with a statistically significant increase in depressive severity, intake episode duration, andhistory of prior suicide attempts.
Discussion: Conclusions from this study are as follows: (1) The presence of one or more subsyndromal manic symptoms is the modal presentation of bipolar MDEs (73%). This is consistent with the reports of other investigators (Benazzi, 2005; Benazzi & Akiskal, 2005; Balázs *et al.*, 2006; Goldberg *et al.*, 2009). (2) The presence of subsyndromal manic symptoms during bipolar MDEs constitutes a prevalent and clinically important form of bipolar mixed state. Key clinical correlates found in this study, such as greater depressive severity at intake, instability of polarity, and heightened suicidal behavior, which are similar to those reported for full manic mixed states (Keller *et al.*, 1986; Hawton *et al.*, 2005; Balázs *et al.*, 2006; Swann *et al.*, 2007; Goldberg *et al.*, 2009). (3) Overt anger/irritability and psychomotor agitation are the most frequent manic symptoms occurring during bipolar MDEs, as reported by others (Benazzi & Akiskal, 2005; Balázs *et al.*, 2006; Goldberg *et al.*, 2009). These pose a particular challenge since they are not only symptoms of mania/hypomania, but have also been used as qualifiers for MDE. (4) Overt anger/irritability and psychomotor agitation, which were analyzed for their separate and combined effects in this study, appear to account for much of the negative findings associated with comorbid subsyndromal manic symptoms, and thus are particularly important markers of a subsyndromal bipolar mixed state. Research is needed on the outcome of antidepressant monotherapy in the presence of subsyndromal manic symptoms. It appears that patients with subsyndromal manic symptoms during bipolar MDEs, particularly overt anger/irritability or psychomotor agitation, should be monitored closely to avoid serious outcomes such as longer affective episodes, polarity switches within the episode, and heightened suicidality and risk of suicide attempts.
Disclosure: L. Judd: None. **P. Schettler:** Part 1: In calendar years 2009 to the present, Dr. Schettler received money from BrainCells, Inc., Methylation Sciences, Novartis BioVentures, and Clintara LLC for statistical consulting services., Part 2: In calendar year 2009, Dr. Schettler received over $10,000 from BrainCells, Inc., for statistical consulting services., Part 3: The money received from BrainCells, Inc., for statistical consulting services constituted more than 5% of personal income in calendar year 2009. **H. Akiskal:** None. **W. Coryell:** None. **J. Fawcett:** None. **J. Fiedorowicz:** None.

171. Use of the Functional Adjusted Clinical States in Bipolar Disorder Methodology in the Lamotrigine Registration Studies in Bipolar Disorder

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Background: All published bipolar maintenance studies that have been used for FDA regulatory registration have employed Kaplan-Meier (KM) survival analytic techniques. KM methodology only provides a single efficacy result of time to a predetermined event (e.g. relapse, intervention, discontinuation etc.). The KM approach is unable to incorporate multiple clinical states, tolerability or functionality into the primary outcome measure. We have

developed the **F**unctional **A**djusted **C**linical **S**tates in **B**ipolar **D**isorder (FACS-BD), a new approach to analyze maintenance studies.

Methods: Data analyses reported here were based on the study reported in Calabrese *et al.* (2003). Patients were randomly assigned to lamotrigine, lithium, or placebo. Our FACS-BD is a multi-state analysis derived from the QTWiST method developed by Gelber and colleagues (e.g., Gelber *et al.*, 1989). Total time spent by each patient on drug was partitioned into separate episodes, each classified into one of seven distinct affective states. These ranged from remission to severe manic, depressed or mixed states, based on published criteria as recommended by the International Society for Bipolar disorders (Tohen *et al* 2010). We used a modification of the KM survival methodology to estimate total duration in days spent in each mood by patients in each medication group during the first year of study treatment.

Results: We first replicated the previously published results using Kaplan-Meier analysis of all-cause discontinuation taking the last rating assessment date as the survival time. This yielded a highly significant effect of medication ($X2 = 11.3$, $df = 2$, $p = 0.0035$). Estimated mean duration was longest on lamotrigine (mean = 207.1 days, SE = 11.5), followed by lithium (mean = 184.0 days, SE = 13.5) and shortest on placebo (mean = 156.5 days, SE = 13.3). Our FACS-BD multi− = state methodology also found longer durations on lamotrigine ($p = 0.01$) and lithium ($p = 0.06$) than on placebo, but most of this added time was spent in a state of mild to moderate depression. Total time asymptomatic was only 20% of the extra time on lamotrigine, and 54% on lithium. Time in remission on both drugs was not significantly longer than on placebo.

Discussion: The multi-state analysis gave a more differentiated picture of clinical outcomes than the Kaplan-Meier analysis of overall time in study. True, those randomized to both lamotrigine and lithium stayed on drug longer than those on placebo, but most of this drug "benefit" was spent experiencing mild to moderate depression. Subsyndromal depression as we defined it did not require dosage adjustment or intervention. Thus, the effects of drug were primarily more time spent in a state of "tolerable depression," not time well. Advantages of the FACS-BD approach over KM include the ability of quantifying different clinical states over a period of time instead of just time to a specified event. In addition FACS-BD can incorporate tolerability weighting and different weighting depending if patients are in full remission with absence of residual symptoms or in remission with some residual symptoms.

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Tohen M *et al.* The International Societyof Bipolar Disorders (ISBD) Task Force on the Nomenclature of Course and Outcomeof Bipolar Disorders Bipolar Disorders 2009 Aug;11(5):453-473.

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172. Can We Detect Bipolar in Youths? Diagnostic Efficiency of Caregiver, Youth, and Teacher Report of Manic Symptoms in Outpatient Settings

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Background: Great progress has been made developing assessment strategies for identification of bipolar disorder and related traits and phenotypes. However, validity of assessment strategies can change markedly between research versus clinically representative samples (Youngstrom *et al.*, 2006). The present study evaluated diagnostic efficiency of measures validated in research clinics when transported to a community setting. The high rates of mood, anxiety, attention problems, and aggressive behavior in a clinically representative sample were expected to increase scores on measures attempting to detect mania, creating more "false positives" and reducing the diagnostic specificity.

Methods: Participants ($N = 894$, ages 5-18 years) were recruited from two settings, a community mental health center (CMHC) and an outpatient academic medical center (AMC) (Youngstrom *et al.*, 2005). Diagnoses were based on the KSADS interview and determined using the LEAD standard (Spitzer, 1983). The criterion diagnosis for the primary analyses of diagnostic efficiency was the presence or absence of bipolar spectrum diagnoses. Analyses compared fourteen combinations of informant and instrument: Parent General Behavior Inventory (Parent Full-length and 10-Mania, Child, Teacher), Mood Disorder Questionnaire (Parent, Child), Young Mania Rating Scale (Parent, Child, Teacher), Child Mania Rating Scale (Parent, Teacher), and Child Behavior Checklist (Parent, Child, Teacher).

Results: Parent report remained valid ($p < .005$) for all parent report measures except the P-YMRS. Youth report tended to discriminate bipolar from nonbipolar cases at better than chance rates, but significantly less well than parent report. Teacher report performed at chance levels. Diagnostic efficiency in the present sample was significantly lower ($ps < .05$) than reported in prior validation studies. Across all six caregiver reported measures investigated, the two bipolar groups (BP I and BP spectrum) were significantly more elevated than the nonbipolar groups (unipolar depression or dysthymic disorder, ADHD or a disruptive behavior disorder), but the bipolar I group never significantly differed from the rest of the bipolar spectrum.

Discussion: The results of this study demonstrated that the effects of sample composition on the diagnostic efficiency performance of instruments could be marked (e.g., Youngstrom, Meyers, Youngstrom, Calabrese, & Findling, 2006). One measure that appeared promising in prior work degraded substantially under more generalizable conditions (P-YMRS), and teacher report failed to ever discriminate bipolar from nonbipolar cases. On the other hand, several parent-reported measures continued to show good discrimination, despite the changes in sample composition and demographics. The measures that continued to discriminate bipolar from other phenotypes may have value as a low-cost method of case-finding or measuring quantitative traits for genetic or imaging studies. Some of these have also already demonstrated excellent sensitivity to treatment effects in clinical trials (Findling, *et al.*, 2003; Youngstrom, Freeman, & Jenkins, 2009).

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Meter: Part 1: N/A, Part 2: N/A, Part 3: N/A, Part 4: N/A, Part 5: N/A. **G. Perez Algorta:** None. **H. Marcinick:** None. **A. Freeman:** None. **O. Meyers:** None. **C. Demeter:** None. **J. Youngstrom:** None. **N. Feeny:** None. **J. Calabrese:** None. **R. Findling:** Part 1: Dr. Findling receives or has received research support, acted as a consultant and/or served on a speaker's bureau for Abbott, Addrenex, Alexza, AstraZeneca, Biovail, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, KemPharm Lilly, Lundbeck, Merck, Neuropharm, Novartis, Noven, Organon, Otsuka, Pfizer, Rhodes Pharmaceuticals, Sanofi-Aventis, Schering-Plough, Seaside Therapeutics, Sepracore, Shire, Solvay, Sunovion, Supernus Pharmaceuticals, Transcept Pharmaceuticals, Validus, and Wyeth., Part 2: Dr. Findling has received income sources and equity of $10,000 per year from Shire., Part 3: None, Part 4: Abbott, Addrenex, AstraZeneca, Bristol-Myers Squibb, Forest, GlaxoSmithKline, Johnson & Johnson, Lilly, Merck, Neuropharm, Otsuka, Pfizer, Rhodes Pharmaceuticals, Schering-Plough, Shire, Supernus Pharmaceuticals, and Wyeth., Part 5: N/A.

173. A Randomized, Placebo Controlled Investigation of Intranasal Oxytocin in Patients with Anxiety

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Background: The peptide neurohormone oxytocin plays a critical role in the regulation of a number of diverse CNS functions which are highly relevant to anxiety including activation of the amygdala and regulation of the HPA axis. Animal studies indicate that oxytocin produces anxiolytic-like effects, and human studies suggest that a single administration of intranasal oxytocin decreases situational anxiety in normal human subjects and decreases amygdala response to social stimuli in patients with social anxiety disorder. As such, there is strong speculation that oxytocin may have therapeutic benefit for anxiety disorders. That said, no controlled clinical trial to date has tested the effects of repeated daily oxytocin in patients with an anxiety disorder. We therefore conducted a randomized, placebo-controlled, pilot study evaluating the efficacy and safety of intranasal oxytocin as adjunctive therapy for generalized anxiety.
Methods: This was a double-blind, randomized cross-over trial. Eligible subjects met DSM IV-TR criteria for generalized anxiety disorder (GAD) and had to have a minimum score of 15 on the Hamilton Anxiety Scale (HAM-A) as well as scores 2 on items 1 (anxious mood) and 2 (tension) at randomization. Other comorbid anxiety disorders were allowed as long as they were less severe than the GAD. Patients already on an anxiolytic medication and those who were not were both eligible as long as there was no change in their medication status or dose for the 3-weeks prior to randomization. No changes in concurrent anxiety medication were allowed during the study. The total study duration was 7 weeks: 3-weeks of daily treatment with adjunctive intranasal oxytocin (titrated to 40 IU twice a day by week 2) and 3-weeks of treatment with adjunctive intranasal placebo. Treatment sequence was randomly assigned and there was one week of washout between treatments. During weekly assessments, HAM-A was measured along with adverse effects and routine blood chemistry. If patients has a least 1 visit on both treatment conditions they were included in an intent-to-treat analysis (LOCF).
Results: Thirteen subjects (7 men & 6 women) met the intent-to-treat criteria (12 subjects completed all visits). Total HAM-A baseline scores at the start of each treatment arm were very similar for placebo and oxytocin (Mean 21.08 and 20.85, respectively). An ANOVA of HAM-A difference scores revealed a drug $\times$ gender interaction that approached significance ($P < 0.078$) which was manifested as distinctly different responses to oxytocin among men and women. In male subjects oxytocin, but not placebo, significantly reduced HAM-A scores below baseline levels beginning week 2. However, in females oxytocin did not significantly improve HAM-A scores, and in fact, tended to produce higher HAM-A scores compared to placebo. Oxytocin was otherwise generally well tolerated by all subjects and there were no significant differences in rates of adverse events or blood chemistry results between placebo and oxytocin.
Discussion: This is the first randomized, placebo-controlled trial of repeated daily oxytocin in a cohort with an anxiety disorder. These preliminary results suggest that there may be significant differences in the response of males and females with anxiety to oxytocin, with men experiencing improvement whereas women do not and may even experience exacerbation. As only one dose of oxytocin was tested in this study, it is not possible to determine if women might require a different dose for anxiolytic effects. However these findings are consistent with existing evidence from other human studies suggesting sexual dimorphism in the central oxytocin system and in the effects of intranasal oxytocin. Further studies are needed to confirm these findings and explore the potential causes for this gender difference.
Disclosure: D. Feifel: Part 1: Addrenex,Astra Zeneca, Bristol Myers Squibb, Sunovion, ELi Lilly, Shire, Forest, Otsuka, Neosync, Part 2: Eli Lilly, Part 3: Eli Lilly, Part 4: Addrenex,Astra Zeneca, Bristol Myers Squibb, Sunovion, ELi Lilly, Shire, Forest, Otsuka, Neosync. **K. Macdonald:** Part 1: Lilly, Janssen, Pfizer, Onu Pharmaceuticals., Part 2: Lilly, Pfizer., Part 3: Lilly., Part 4: Goodenough fund for oxytocin research., Part 5: NA. **R. McKinney:** None. **N. Heisserer:** None. **V. Serrano:** None.

174. Comparison of High-Dose Escitalopram, Bupropion, and their Combination from Treatment Initiation in Major Depressive Disorder: A Double-Blind Study

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Background: The common treatments for major depressive disorder (MDD) are plagued with a slow onset of action and a low remission rate. In the presence of inadequate responses, drug combinations are commonly used. Two prior double-blind studies using mirtazapine combinations from treatment initiation produced significantly greater responses than antidepressants used in monotherapy (Eur Neuropsychopharmacol 19:457, 2009; Am J Psychiat 167:281, 2010). The present study was first designed to examine the effectiveness of a different combination of antidepressants from treatment initiation. The escitalopram and bupropion combination was chosen based on their synergy between the serotonin and norepinephrine systems documented in electrophysiological experiments in the rat brain (J Psychopharmacol 24:39, 2010). The current study was also designed to determine if the antidepressant response could be hastened by rapidly titrating both antidepressants and using regimens of escitalopram above its highest recommended dose of 20 mg/day, as suggested in a preliminary open label study (J Psychiat Pract 15:337, 2009).
Methods: Patients with MDD ($n = 241$) in three centers, who had a minimum score of 22 on the Montgomery Asberg Depression Rating Scale (MADRS), were randomized to receive escalating doses at weekly intervals of escitalopram (10-40 mg/day), bupropion (150-450 mg/day), or their combination, according to tolerability and/or achievement of remission status (score of 7 or less on the 17-item Hamilton Depression Scale; HAMD). Patients were assessed using the HAMD, the MADRS, and the Clinical

Global Impression-Severity (CGI-S) and CGI-improvement (CGI-I) scales, and patients rated themselves using the Quick Inventory of Depression Symptoms-self report, the Quality of Life Inventory, the Social Adjustment Scale, and the Change in Sexual Functioning Questionnaire. Assessments were done weekly for the first 4 weeks and at weeks 6, 8, 10, and 12.

Results: The sample was composed of 66% females and did not differ significantly at baseline on any of the demographics between the three groups (age, gender, race, education, marital status, employment status, HAMD, MADRS, CGI-S). The overall means ($\pm$ SD) were for: age 41 ± 11 years; HAM-D17, 20 ± 5; MADRS, 29 ± 5, CGI-S, 4.6 ± 0.1). The mean daily doses at study exit were: 30 mg for escitalopram, 360 mg for bupropion and 31 and 358 mg in the combination group. The dropout rates at week 12 were not statistically different: 25% for escitalopram, 35% for bupropion, and 30% for the combination (Chi-square = 1.98, df = 2, P = 0.37). There was a significantly greater decrease in scores in the escitalopram, but not in the combination, when compared to the bupropion group at week 12 for the HAMD17 (F = 3.68, df = 2, 238, P = 0.03), the MADRS (F = 4.00, df = 2, 238, P = 0.04), the CGI-S (F = 3.42, df = 2, 237, P = 0.03), and the CGI-I (F = 3.24, df = 2, 237, P = 0.04). The proportion of patients achieving a score of 2 or 1 on the CGI-I were 63% on escitalopram, 44% on bupropion, and 55% on the combination (Chi-square = 6.34, df = 2, P = 0.04). There was a greater number of remitters on escitalopram (54%) than on bupropion (33%), but not on the combination (42%): Chi-square = 7.67, df = 2, P = 0.02. Complete analyses, including onset of action, self-ratings, and site comparisons, will be presented at the meeting.

Discussion: Both antidepressants, whether they were used in monotherapy or in combination, were relatively well tolerated using a rapid escalation, at least based on dropout rates. Higher doses than the maximal recommended dose of escitalopram clearly outperformed optimal doses of bupropion in attenuating depressive symptoms; in contrast, two prior double-blind studies using conventional doses of these two medications showed that they had similar effectiveness (J Clin Psychiat 67:736, 2006). The combination of escitalopram and bupropion was not significantly more effective than any of the two drugs used alone in the overall analysis. These results suggest that the use of higher than normal doses of escitalopram is well tolerated and yields a similar remission rate when compared to that achieved with the concomitant use of bupropion from the start.

Disclosure: P. Blier: Part 1: Astra Zeneca, Bristol Myers Squibb, Eli Lilly, Euthymics, Janssen, Lundbeck, Merck, Pfizer, Schering-Plough, Servier, Takeda, Wyeth, Part 2: None, Part 3: None, Part 4: Astra Zeneca, Bristol Myers Squibb,, Janssen, Lundbeck, Merck, Pfizer, Schering-Plough, Servier, Wyeth, Part 5: No. **P. McGrath:** None. **R. Bergeron:** Part 4: Lundbeck/Takeda, Wyeth, Part 5: No. **J. Stewart:** Part 1: Pfizer.

175. Correlates of Ziprasidone Adherence in the Maintenance Treatment of Bipolar Disorder: Analysis of STEP-BD Data

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Background: Medication adherence is an ongoing concern in the treatment of bipolar disorder and recent research suggests that, across medications, nonadherence averages 15%. Ziprasidone is approved for maintenance therapy of bipolar disorder as adjunctive therapy with lithium or valproate, yet no data exist regarding profiles of nonadherent patients or factors that are associated with nonadherence in ziprasidone treatment.

Methods: Data from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) were used to identify 282 patients who had received ziprasidone as part of the naturalistic study. Patients were followed for up to four years and self-reported their previous two-week adherence during at least quarterly visits. In keeping with previous studies, missing 25% or more of the total prescribed dose of ziprasidone over the two weeks preceding an appointment was classified as nonadherence. A recursive receiver operating characteristic (ROC) approach was applied to detect the presence of profiles associated with nonadherence. This analysis was followed by a mixed random effects regression analysis (including all visits from patients) with adherence as a dependent measure and reported adverse events, and demographic variables as predictors.

Results: Of the 282 patients, 171 patients received ziprasidone as adjunctive therapy with lithium or valproate (the remainder were on ziprasidone without either agent). Nonadherence with ziprasidone adjunctive therapy was low, with only 4% of patients reporting nonadherence across 1,861 total patient visits. ROC analyses did not suggest any profile associated with nonadherence (P > .01). Adverse events (constipation, diarrhea, dry mouth, headache, memory loss, sedation, sexual dysfunction, or tremor) did not exhibit any association with nonadherence across episodes. Regression analyses revealed that nonadherence had a significant inverse association with age, but a positive association with suicide attempt history and alcohol abuse among patients receiving ziprasidone as adjunctive therapy.

Discussion: Ziprasidone has a low nonadherence rate (4%) in the maintenance treatment of bipolar disorder when used as adjunctive therapy with lithium or valproate, which compares favorably to overall medication nonadherence (15%) in STEP-BD patients. Nonadherence with ziprasidone adjunctive therapy was not significantly associated with adverse events. The findings suggest that bipolar disorder patients who are younger and have a history of alcohol and suicide attempts appear to warrant additional efforts to ensure adherence with ziprasidone adjunctive therapy.

Disclosure: J. Brooks: Part 1: Dr. Brooks is on the Speaker's Bureau for Merck and Sunovion., Part 2: Sunovion, Part 4: This work was supported by an unrestricted Investigator Initiated Grant to Dr. Brooks.

176. Neural Activity in Visual Cortical Areas during the Processing of Emotional Expressions Predicts Antidepressant Response to the Anticholinergic Scopolamine

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Background: Acetylcholine (ACh) plays a major role in working memory, likely through influences on stimulus processing. ACh muscarinic receptors are hyper-responsive in individuals with major depressive disorder (MDD) and these cholinergic receptors may modify stimulus processing and thus alter the processing of emotional stimuli in MDD. Moreover, the antimuscarinic agent scopolamine has been shown to produce antidepressant effects. The goal of this study is to determine if the functional brain response during the processing of stimuli in a working memory task may effectively predict the antidepressant response to scopolamine.

Methods: Unmedicated outpatients with MDD (N = 15) participated in a double-blind, placebo controlled, crossover clinical trial of scopolamine. Following a single-blind placebo session, participants were randomized into either S/P or P/S (S = 3 sessions of i.v. scopolamine (4ug/kg); P = 3 sessions of i.v. placebo). Depression severity was determined prior to each infusion using the Montgomery & Asberg Depression Rating Scale (MADRS).

Following infusions in sessions 1, 2, and 5 (this ensured that we would obtain baseline data during session 1, and randomly obtain placebo and scopolamine in sessions 2 and 5) patients performed a working memory task in a 3T GE scanner. During the task, a picture of a face was shown (encoding) followed by a delay component (maintenance) and another picture of a face (test/retrieval). Trials were separated by a 15 sec inter-trial interval. Subjects were instructed in blocks of 8 trials to attend to either the identity or the emotional expression of the face during the encoding period, and indicate a match or non-match during the test period based on the attended feature. Antidepressant response was calculated as percent change in MADRS from baseline to study end. Multiple regression was used to calculate the BOLD response during each of the three task components, for each of the two WM conditions. To control for multiple comparisons, overall task responsive brain regions ($p < 0.05$ whole brain corrected) were identified and a mask was created to restrict subsequent analyses. Baseline (session 1) BOLD signal for each of the six task conditions was correlated with percent change in MADRS within brain regions that responded to the overall working memory task (SVC $p < 0.05$). Correlations between treatment response and change in BOLD (drug-placebo) also were conducted. These analyses were restricted to task-responsive brain regions (SVC $p < 0.05$) and were conducted only in task conditions with significant baseline correlations. Reaction time (RT) and accuracy data were collected.
Results: Participants showed a significant reduction in MADRS score from baseline to study end ($p < 0.001$), with improvement ranging from a 10% to a 100% reduction in score. Significant correlations between clinical response magnitude and BOLD signal were observed in the middle occipital visual cortex for both the encoding (left, $r = -.85$; right, $r = -.77$) and retrieval (left, $r = -.87$; right, $r = -0.81$) components of the task, selectively during the emotion working memory task. No correlation was observed between clinical resjponse and BOLD activity during the identity task. A significant correlation between treatment response and change in BOLD (drug-placebo) was observed during emotion encoding ($r = +.74$) in an area of left middle occipital cortex that overlapped spatially with the correlation seen at baseline. A significant task $\times$ drug interaction ($p < 0.05$) was seen in RT, where a relative decrease in RT selectively during the emotion task and a relative increase during the identify task were observed under scopolamine relative to placebo.
Discussion: The results indicate that baseline levels of neural activity in visual processing areas reflect potential for response to treatment with scopolamine. The cortical regions that show baseline predictive value for treatment response also respond to cholinergic modulation, which argues that baseline differences are cholinergically mediated. Baseline levels of neural activity to emotional stimuli in visual processing areas may provide a putative biomarker of treatment response to the anticholinergic agent scopolamine in patients with MDD.
Disclosure: M. Furey: Part 1: Dr Furey is listed as a co-inventor on a patent application for the use of scopolamine in major depression. Dr. Furey has assigned her rights in the use-patent for scopolamine as an antidepressant agent to the U.S. government but will share a percentage of any royalties that may be received by the government. **E. Frankel:** None. **E. Hoffman:** None. **A. Speer:** None. **W. Drevets:** Part 1: Dr. Drevets is listed as a co-inventor on a patent application for the use of scopolamine in major depression. Dr Drevets has assigned his rights in the patent to the U.S. government but will share a percentage of any royalties that may be received by the government. Dr. Drevets also has consulted for Pfizer Pharmaceuticals and for Johnson and Johnson. **C. Zarate, Jr:** Part 1: Dr. Zarate is listed as a co-inventor on a patent application for the use of ketamine in major depression. Dr. Zarate has assigned his rights in the patent to the U.S. government but will share a percentage of any royalties that may be received by the government.

177. Effect of Lithium and Valproate on Brain Activation Patterns in fMRI in a Working Memory Paradigm in Bipolar I Patients

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Background: Patients with bipolar disorder have been shown to have different activity patterns in fMRI when compared to healthy controls; specifically, they show increased activation in limbic and para-limbic areas, whereas they show decreased activity in working memory-related areas. The degree to which pharmacological treatment determines these alterations is hard to gage, given that most studies have been done on patients already receiving such treatments. There are very limited and often contradictory data on the subject.
Methods: Aim: We seek to identify differences and identify the role of treatment in neurofunctional response in patients with bipolar disorder type I, compared to controls, specifically while challenged with working memory tasks. Thirty-three (33) euthymic patients with bipolar disorder type I and ten (10) controls were evaluated in a cross-sectional study; 13 of them were on treatment with lithium, 9 on valproate and 10 without treatment for at least 2 months prior to the study. Correlation between functional Magnetic Resonance (fMRI) BOLD signal and working memory processes.
Results: There were no significant differences between the groups in demographic or clinical variables except for YMRS score. Patients and controls demonstrated significantly different patterns of brain activation in anterior cingulated (p:0.05) during working memory task. There were no differences in the angular gyrus, fronto-orbital cortex and frontal lobe. There were no difference in activation patterns in fMRI between patients treated with valproate or lithium and patients without pharmacologic treatment.
Discussion: There are statistically significant differences in the anterior cingulate BOLD (Blood oxygen level dependent) signal between patients with Type I Bipolar disorder compared to controls. There were no other differences in the studied regions. Treatment with valproate and lithium did not play a role in those differences.
Disclosure: C. Lopez-Jaramillo: None. **J. Lopera-vasquez:** None. **J. Delgado:** None. **S. Rascovsky:** None. **J. Escobar:** None.

178. Genetic Ancestry among Self-Reported African-Americans and Pharmacogentic Response to SSRI Treatment of Major Depression

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Background: The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Study currently remains the largest and most comprehensive clinical trial investigating beneficial response to antidepressant medicine. The study boasts several strengths including a racially diverse cohort with an adequate representation of self-identified Black/African Americans (AA), who have traditionally been underrepresented in similar clinical trials. However, despite groundbreaking and clinically useful insights into the general responses of depressed patients to antidepressant medication, findings from the study have failed to shed much light on the relatively lower rates of remission among AA compared to white patients. This disparity has been the subject of much speculation, spanning a range of explanations from socioeconomic and clinical disadvantage (Lesser *et al*., 2007) to possible race-associated genetic influences (McMahon *et al*., 2006), all of which have had plausible but inconclusive empirical support. To address some of the limitations of the previous studies we included in our analyses genetic ancestry data in conjunction with the other sociological and clinical variables. The objective was to determine

(1) the extent to which genetic ancestry was associated with self-reported race, and (2) whether there was an independent association of genetic ancestry with initial improvement in depression in response to antidepressant treatment, after adjusting for potential confounds of self-reported race and other demographic, psychosocial and clinical covariates.
Methods: The analyses focused on a STAR*D sub-sample of depressed patients who received the same type of treatment through the first level. This group comprised 313 self-reported AA, 1526 self-reported Whites and 100 self-reported Asian/Pacific Islander, Native American, "other" or "mixed race." Eight African-Americans and 247 Whites reported Hispanic ethnicity. Using multidimensional scaling (MDS), we extracted four clusters associated with population ancestry from genotyped data using the publicly available software (PLINK), with the second cluster (C2) highly correlated with self-reported African Ancestry ($r = 0.86$). The outcome measures were change scores in measures of depression from baseline to end of treatment (level 1), using the Hamilton Rating Scale of Depression-17 item (HRSD-17), the Inventory of Depressive Symptomatology -30 item (IDS-30), and the Quick Inventory of Depressive Symptomatology – Clinician rated(QIDS-C). We then ran general linear models for each outcome measure separately, first unadjusted with C2 as the predictor, and then with the relevant covariates included.
Results: All the unadjusted models using genetic ancestry as the sole predictor variable were statistically significant at $p < .05$. However, adjusting for the covariates - particularly medical conditions and household income - virtually eliminated the contribution of C2 to change scores on the HRSD-17, QIDS-C and QRCS-30. In the case of AA and whites only, self-reported race and C2 were highly correlated ($r = 0.96$) Deleting self-reported race from the models led to a marginally significant effect of C2 ($.01 < p < .05$) even after all environmental and clinical covariates were included in the model. Independent of self-reported race or genetic ancestry, the biggest influences on improvement in scores the full model were female gender, higher household income, and better baseline scores on depression and absence of general medical conditions.
Discussion: The preliminary results suggest a relatively minor and clinically insignificant contribution of genetic, relative to socio-economic and clinical influences on the Black-White disparity in initial therapeutic response to antidepressants. The socio-demographic and economic influences suggest issues related to compliance with the regimen. The higher levels of baseline depression and comorbid medical conditions among the African American patients may point to possible biases inherent in the process of self-selection into a pharmacological treatment study of depression.
Disclosure: E. Murphy: None. **F. McMahon:** None.

179. 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: S. Stroup: Part 1: consultant for Lilly and Janssen, Part 2: None, Part 3: None, Part 4: None, Part 5: NOne. **R. Hamer:** Part 1: Abbott-Served on multiple DSMBs (formerly Solvay) Acadia - Advised on the design of a clinical trial Allergan-Served on a DSMB Alpharma-Served on a Mock Advisory Panel (3 meetings) AstraZeneca-Statistician on a UNC contract for a clinical trial Cenerx-Advised on the design and statistical analyses plan of a clinical trial Corcept-Consultant in the design and analysis of multiple clinical trials Eli Lilly- Served on DSMBs EnableMD-Served on an Advisory Board Epix-Advised on the design and analysis of multiple clinical trials J&J –Consulting statistician on epidemiological analyses of data from a VA database NeuroPharmaBoost- Consult on the Design of a Clinical Trial for an antidepressant: unbilled unpaid Novartis-Served on Advisory Board Pepper-Hamilton- Advised lawyers regarding a lawsuit (1 teleconference) unbilled unpaid Pfizer-Served on multiple DSMBs PureTechVentures-Consult on the design of a Clinical Trial for an antidepressant SAS Institute-Taught several seminars on statistics using SAS for SAS Institute Schwarz-Served on multiple DSMBs

Solvey-Served on a DSMB Sanofi-Aventis -Consulted on the design of a clinical trial Takeda- Consulted on the design of a clinical trial Winston & Strawn- Expert witness in lawsuit involving Forest, Lundbeck, Sun and Caraco Winston & Strawn- Expert witness in lawsuit involving Teva, Barr, Mylan, Eurand, Cephalon, Anesta Wyeth-Served on an Advisory Board, Part 2: None, Part 3: Additionally, I or my wife own stock in Bristol-Myers Squibb, Amgen, Lilly, Genetech, Proctor and Gamble, Sepracor. Additionally, my wife is retired from Bristol-Myers Squibb has stock options which currently have negative value, Part 4: None, Part 5: Not Applicable. **N. Ray:** None. **S. Esscok:** None. **J. Lieberman:** Part 1: Advisory board on Alkermes, Bioline, Intracellular Therapies, Pierre Fabre, Psychogenics, Lilly I receive no direct financial compensation or salary support for participation in research, consulting, or advisory board activities, Part 4: Allon GlaxoSmithKline Lilly Merck Novartis Pfizer F. Hoffmann-La Roche LTD Sepracor (Sunovion) Targacept, Part 5: Not Applicable.

180. Gender Differences in a Placebo-Controlled Trial of Fluoxetine for Body Dysmorphic Disorder

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Background: Body dysmorphic disorder (BDD) is a severe and common disorder that consists of distressing or impairing preoccupation with nonexistent or slight defects in one's physical appearance. We previously reported results from the only placebo-controlled pharmacotherapy study of BDD, which found that fluoxetine was significantly more efficacious than placebo for BDD. However, the relationship of gender to treatment outcome was not examined in detail. Some studies of depressive and anxiety disorders have found gender differences in treatment outcome whereas others have not. To our knowledge, no prior report has focused on the relationship of gender to treatment outcome in BDD.
Methods: After 1 week of single-blind placebo, 67 patients (46 females, 21 males) with DSM-IV BDD (including patients with delusional BDD beliefs) were randomized to 12 weeks of double-blind treatment with fluoxetine or placebo. Generalized linear mixed modeling tested whether gender and group (fluoxetine vs placebo) were related to change in BDD severity (BDD-YBOCS), depression severity (HAM-D), psychiatric symptom severity (BPRS), and delusionality of BDD beliefs (Brown Assessment of Beliefs Scale [BABS]). Response of BDD to treatment (30% or greater improvement in BDD-YBOCS score) was additionally evaluated in relation to change on other measures.
Results: Regarding degree of improvement in BDD symptoms on the BDD-YBOCS, women had greater improvement in BDD severity than men with treatment (fluoxetine or placebo; $p = 0.023$). However, the proportion of subjects who were classified as treatment responders did not significantly differ by gender($p = 0.417$); 50% of women vs 60% of men had response of BDD symptoms to fluoxetine ($p = 0.595$), and 14% of women vs 27% of men had response of BDD symptoms to placebo ($p = 0.338$). For the entire sample, HAM-D and BPRS scores decreased significantly more with fluoxetine than with placebo ($p = 0.01$ and $p = 0.03$, respectively), as previously reported. Men and women did not significantly differ in improvement on the HAM-D ($p = 0.317$) or BPRS ($p = 0.483$). However, a significant gender $\times$ BDD response $\times$ time interaction was found for depression severity ($p = 0.020$), such that females whose BDD symptoms responded to treatment showed greater improvement in HAM-D score than female non-responders, whereas male non-responders showed greater improvement in HAM-D score than male treatment responders. A significant gender $\times$ BDD response $\times$ time interaction was also found for the BPRS ($p = 0.011$). Women whose BDD symptoms responded to treatment had greater improvement on the BPRS than male treatment responders; however, female non-responders and male non-responders did not significantly differ. No significant group or gender differences were found for delusionality of BDD beliefs as assessed by the BABS.
Discussion: Women had significantly greater improvement than men in BDD symptom severity with treatment. However, BDD response rates to either fluoxetine or placebo did not significantly differ by gender. Gender differences in improvement on the HAM-D and BPRS were related to response of BDD to treatment. These preliminary results suggest that contrary to some studies of gender-related response rates to SSRI treatment in other disorders, women with BDD do not have a higher response rate to fluoxetine treatment than men with BDD. However, change in depressive and other psychiatric symptoms is more closely associated with BDD treatment response for women than for men. Although statistical power was limited, these post-hoc exploratory analyses may inform future research on the relationship of gender to treatment outcome in BDD.
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181. Lamotrigine in Bipolar Disorder, Depressed or Mixed Phase and Cocaine Dependence

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Background: Bipolar disorder is associated with the highest rates of drug and alcohol dependence of any Axis I illness. Use of cocaine is particularly common in this population. However, very limited data are available on the treatment of this large and clinically important important dual diagnosis population.
Methods: A 10-week, randomized, double-blind, placebo-controlled trial of lamotrigine (titrated up to 400 mg/day) was conducted in 120 adult outpatients with bipolar disorder, depressed or mixed mood state, and cocaine dependence. Participants were recruited from the community using paid and free advertising, and referrals from providers. All participants provided written, UT Southwestern-approved informed consent. Cocaine use was quantified weekly both by participant-report using the time line follow-back method and with urine drug screens. Mood was assessed with the Hamilton Rating Scale for Depression (HRSD), Inventory of Depressive Symptomatology-Self Report (IDS-SR) and Young Mania Rating Scale (YMRS). Side effects were assessed with The Psychobiology of Recovery in Depression (PRD-III) Somatic Symptom Scale. Cocaine craving was assessed with the Cocaine Craving Questionnaire (CCQ). Data were analyzed using a random regression analysis that used all available data from participants with at least one post-baseline assessment ($n = 112$).
Results: Lamotrigine and placebo groups were similar demographically (age 45.1 ± 7.3 vs. 43.5 ± 10.0 years, 41.8% vs. 38.6% women, 54.5% vs. 50.9% bipolar I, 89.1% vs. 91.2% depressed). Dollars

spent on cocaine showed a significant initial (baseline to week 1) ($p = 0.01$) and growth effect (weeks 1-10) ($p = 0.05$) favoring lamotrigine. Urine drug screens, and mood symptoms were not significantly different between groups. Side effects and survival in the study were not significantly different in the two groups.
Discussion: Few positive trials of medications for cocaine use, other than stimulant replacement, have been reported and, to our knowledge, none have been reported in bipolar disorder. The reduction in amount of cocaine use with lamotrigine suggests that a standard treatment for bipolar disorder may also reduce the amount of cocaine use in this population. A limitation of the study was the weekly assessment of urine drug screens that decreased the ability to detect between-group differences on this outcome.
Disclosure: E. Brown: Part 1: Grants from AstraZeneca, GlaxoSmithKline, Forest Research Institute, Sunovion, Part 4: Grants from AstraZeneca, GlaxoSmithKline, Forest Research Institute, Sunovion. **P. Sunderajan:** Part 1: Grants from Bristol-Myers Squibb; Lilly USA, LLC; Takeda Pharmaceuticals North America, Inc., Part 4: Grants from Bristol-Myers Squibb; Lilly USA, LLC; Takeda Pharmaceuticals North America, Inc. **L. Hu:** None. **S. Sowell:** None. **T. Carmody:** Part 1: Cyberonics, Inc.

182. Oral Naltrexone Alters the Subjective but not the Physiological Effects of Oral d-Amphetamine in Humans

Sandra D. Comer*, Phillip A. Saccone, Perrine Roux, Jermaine D. Jones, Ziva D. Cooper, Suzanne K. Vosburg, Maria A. Sullivan, Eric Rubin, Jeanne M. Manubay, Shanthi Mogali, Margaret Haney, Richard W. Foltin

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Background: Although amphetamine dependence is a serious public health concern, no medications are approved by the FDA for treating this disorder. Recent studies have suggested that oral naltrexone (NTX) is effective in reducing the subjective effects of amphetamine in humans. The purpose of this study was to further evaluate the ability of NTX to alter the cardiovascular and subjective effects of amphetamine compared to cocaine.
Methods: Non-treatment seeking cocaine users ($N = 6$) participated in this randomized, within-subject, placebo-controlled, double-blind inpatient study. Over 10 sessions, with at least one day off in between, participants received 0, 12.5, or 50 mg of NTX one hour prior to stimulant administration. Oral d-amphetamine (0, 10, and 20 mg) was administered in ascending order using a 60-min interdose interval. Smoked cocaine (0, 12.5, 25, and 50 mg) was administered in ascending order using a 14-min interdose interval. Subjective and cardiovascular effects were measured before and repeatedly after drug administration.
Results: Both amphetamine and cocaine produced dose-related increases in subjective and cardiovascular effects under placebo NTX conditions. Cocaine and amphetamine produced similar cardiovascular effects, but the subjective effects of amphetamine were less robust. NTX 12.5 and 50 mg administered in combination with the highest dose of amphetamine reduced ratings of "High," "Liked the choice," "Of high quality," and "Potent" compared to placebo NTX (all $P < 0.01$). NTX did not alter any of the subjective effects of smoked cocaine. NTX did not alter the cardiovascular effects of either amphetamine or cocaine.
Discussion: These preliminary results demonstrate that NTX attenuates the positive subjective effects of amphetamine, but has no effect on cocaine in cocaine abusing participants. Future studies should evaluate both oral and sustained-release NTX as potential therapeutic agents for amphetamine dependence. Interestingly, the data also implicate the opioid system in modulating the abuse potential of amphetamine, but not cocaine.
Disclosure: S. Comer: None. **P. Saccone:** None. **P. Roux:** None. **J. Jones:** None. **Z. Cooper:** None. **S. Vosburg:** None. **M. Sullivan:** None. **E. Rubin:** None. **J. Manubay:** None. **S. Mogali:** None. **M. Haney:** None. **R. Foltin:** None.

183. Effects of D-Amphetamine on Responses to Emotional Stimuli

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Background: Stimulant drugs of abuse produce feelings of euphoria, but in addition to this, they may also enhance the value of positive stimuli in the environment. Drug users report that drugs enhance positive external stimuli (e.g. parties) and that this is a major motive for drug use. Preclinical studies support the idea that stimulant drugs enhance the value of positive stimuli. However, the effect of drugs on responses to emotional stimuli in the environment has rarely been investigated in controlled studies in humans, and has not been distinguished from direct effects of drugs on mood (i.e. euphoria). Thus in the current study, we examined effects of *d*-amphetamine, a prototypic stimulant, on both self-reported mood and on reactivity to emotional stimuli in healthy volunteers. We predicted amphetamine would enhance reactivity to pleasant stimuli, particularly stimuli with social content, and that these effects would occur independently of the typical euphoric effects of the drug.
Methods: Over three sessions, 36 healthy normal adults received placebo, *d*-amphetamine 10mg, and 20mg under counterbalanced double-blind conditions. At each session emotional reactivity to standardized positive, neutral and negative pictures with and without social content was quantified using both self-reports and activity in facial muscles sensitive to emotional state. Typical cardiovascular and subjective drug effects were also measured.
Results: As expected, amphetamine produced euphoria, arousal, feelings of drug effect and increased blood pressure. Notably, amphetamine also enhanced self-reported positive reactions to all pictures, and psychophysiological reactions to positive pictures. These effects were not mediated by drug-induced euphoria or arousal. Contrary to our hypothesis, amphetamine did not differentially alter responses to images with social content.
Discussion: This study demonstrates a novel, potentially reinforcing effect of stimulant drugs that is distinct from more typically measured euphorigenic effects, and suggests new areas of research in stimulant abuse risk factors.
Disclosure: M. Wardle: None. **H. de Wit:** Part 4: Grant from Unilever.

184. The Effects of the NK1 Antagonist Aprepitant on Opioid Withdrawal in Patients Maintained on Methadone

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Background: Although opioid substitution therapy is an effective clinical tool used to manage opioid abuse and dependence, concerns regarding current FDA-approved medications have led to a search for efficacious, non-opioid medications. Preclinical data indicate that Neurokinin 1 (NK1) receptor activity may modulate opioid withdrawal and reward. This pilot investigation is the first placebo-controlled, clinical studies evaluating the effects of the NK1 antagonist aprepitant (Emend®) on this topic.
Methods: Fifteen patients (11 men and 4 women; 6 African-Americans, 5 Caucasians and 4 Latinos) with opioid dependence maintained on daily doses of methadone (40–100 mg/day) participated in a study to assess the effects of aprepitant (80 mg)

on methadone withdrawal. This outpatient, placebo-controlled, double-blind, crossover study consisted of non-treatment (placebo) and treatment (aprepitant) arms. Half of the participants received aprepitant first. Experimental assessments occurred on Days 1-3 (placebo or aprepitant) and again on Days 8-10 (placebo or aprepitant). On the first two days (Days 1, 2 and 8, 9) of each testing period, participants received study medication (placebo or aprepitant) at 9 am and their usual dose of methadone at 10 am. On the third day of each testing period (Days 3 and 10), participants consumed their study medication (placebo or aprepitant) at 9 am but did not receive their methadone dose until 4 pm.The effects of aprepitant were analyzed in 2 models. The first model evaluated the interaction of aprepitant with acute methadone on the second days of treatment (Days 2 and 9). The second model assessed the effects of aprepitant on methadone withdrawal measured on the 3rd day of treatment (Days 3, 10). Primary outcome measures were: effects of aprepitant on the subjective and objective assessments of opioid withdrawal (measured on Days 3 and 10), along with subjective effects of methadone in combination with aprepitant (measured on Days 2 and 9). Outcome measures were analyzed using repeated-measures ANOVA.

Results: Statistical analyses indicate that aprepitant reduced COWS scores during the 6 hour period during which methadone was withheld ($p = 0.10$). Similarly, when administered with methadone, participants reported less desire to use methadone ($p = 0.09$), less opioid craving ($p = 0.16$), and less opioid withdrawal ($p = 0.02$). Interestingly, 1 hr after aprepitant and methadone were co-administered, ratings of "High" ($p = 0.05$) and "Liking" ($p = 0.03$) increased significantly.

Discussion: These data tentatively suggest that aprepitant has some ability to alleviate methadone withdrawal. Aprepitant also appears to potentiate some methadone effects. Since few of the differences between aprepitant and placebo reached statistical significance, these data should only be viewed as preliminary. Findings from other studies indicate that higher doses of aprepitant (200 mg) may be more clinically effective (Walsh, 2011). Further clinical investigations are needed in order to determine whether aprepitant is useful for alleviating opioid withdrawal symptoms.

Disclosure: J. Jones: None. **T. Speer:** None. **E. Nunes:** None. **S. Comer:** None. **S. Ross:** None. **J. Rotrosen:** None. **M. Reed:** None.

185. Naltrexone Reduces Women's Weight Gain in Smoking Cessation at Six Month Follow-Up

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Background: Successful smoking cessation is often accompanied by weight gain. This paradoxical effect of quitting smoking is particularly salient for women who are often are more concerned than men about gaining weight during a smoking quit attempt. None of the approved pharmacotherapies for nicotine dependence reduce the weight gain associated with smoking abstinence.

Methods: This investigation examined the effects of the opioid receptor antagonist naltrexone compared to placebo on weight gain in a combined patient sample from three randomized, placebo-controlled clinical trials conducted at the University of Chicago (King *et al.*, 2011 under review) and Yale University (O'Malley *et al.*, 2006; Toll *et al.*, 2010). Across trials, smokers were randomized to daily naltrexone (25, 50, or 100 mg) or placebo for intervals ranging from 6-26 weeks, with all receiving short-term nicotine patch for up to 6 weeks and behavioral counseling for up to one month after the quit date.

Results: The analysis sample consisted of participants who were biochemically-verified as abstinent from smoking six months after the quit date. This included 23% (N = 198/872, 108 women) of the originally enrolled sample across studies. Analyses examined medication main effects and the interaction of medication by sex, while controlling for study site. Results showed that, for women, naltrexone (vs. placebo) significantly reduced the number of pounds gained at six months (naltrexone, 7.23 ± 1.25 SEM, placebo 11.07 ± 1.28 lbs), but this was not the case for men (10.29 ± 1.01 vs. 8.46 ± 1.25 lbs, respectively; med × sex, $p = 0.01$). Also, for women, naltrexone reduced clinically significant weight gain defined by the FDA as ⩾7% increase of baseline body weight, with 36% of women in the naltrexone group and 49% in the placebo group meeting this criteria at six months. However, this was not the case for men, as 40% of men in the naltrexone group vs. 29% in the placebo group met this criteria (med × sex, $p < .05$). Analyses repeated on the two datasets (N = 156; King *et al.*, 2011 under review; O'Malley *et al.*, 2006) with shorter-term naltrexone or placebo treatment of either six or eight weeks confirmed these results, as women, but not men, who received naltrexone had less weight gain (naltrexone, 8.56 ± 1.46, placebo 12.17 ± 1.53 lbs; med × sex, $p < .05$) and a lower prevalence of meeting the FDA significant weight gain criteria at six months (38% in naltrexone group vs. 56% in placebo group; med × sex, $p < .01$).

Discussion: The results provide support for naltrexone as the first pharmacotherapy to significantly reduce women's long-term weight gain in smoking cessation.

Disclosure: A. King: None. **D. Cao:** None. **L. Zhang:** None. **S. O'Malley:** None.

186. Effects of MDMA on Social and Emotional Processing in Humans

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Background: MDMA (±3,4-methylenedioxymethamphetamine, 'ecstasy') is reportedly used recreationally because it increases feelings of empathy, sociability, and interpersonal closeness. However, empirical information about these so-called "empathogenic" effects is lacking. We are investigating the acute effects of MDMA on social and emotional processing in healthy human volunteers, using controlled laboratory procedures.

Methods: MDMA users (N = 28) participated in a 4-session, within-participant, double-blind study, in which they received oral MDMA (0.75, 1.5 mg/kg), intranasal oxytocin (20 IU), or placebo. Oxytocin was selected as an active control drug because MDMA reportedly increases oxytocin levels in the brain, and because of similarities in its pro-social behavioral effects. The primary outcome measures included emotion recognition, emotional responsiveness, and sociability (desire to be with others). Cardiovascular and subjective effects were also assessed. Subjects were tested individually.

Results: As expected, MDMA dose-dependently increased heart rate and blood pressure and feelings of euphoria (e.g., 'High' and 'Like Drug'). On measures of social function, MDMA impaired recognition of fearful facial expressions, and the larger dose (1.5 mg/kg) increased feelings of sociability and increased desire to be with others, compared to placebo. Interestingly, MDMA also increased ratings of loneliness, perhaps related to the individual testing environment ($p < 0.05$ for all comparisons). Oxytocin did not produce significant effects on any dependent measure.

Discussion: These data indicate that MDMA not only increases euphoria but also feelings of sociability, perhaps by reducing sensitivity to subtle signs of negative emotions. The increase in

desire to socialize and the reduced reactivity to negative emotions in others may contribute to its attractiveness to recreational users. This research is supported by NIDA R01 DA02812.
Disclosure: M. Kirkpatrick: None. **M. Wardle:** None. **R. Lee:** None. **H. de Wit:** None.

187. Baclofen as a Novel Pharmacotherapy for Alcohol Dependence: Preliminary Findings from a Human Laboratory Double-Blind Placebo-Controlled Randomized Study

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Background: Prior studies have identified the selective $GABA_B$ receptor agonist baclofen as a possible novel pharmacotherapy for alcohol dependence (AD). We conducted a human laboratory study to investigate putative biobehavioral mechanisms by which baclofen reduces drinking [attenuating urges to drink, decreasing attention to alcohol cues, attenuating pleasurable affects of alcohol and/or increasing the aversive effects of alcohol, attenuating pleasurable effects of slip]; by ways of a cue-reactivity (CR) experiment, followed by an alcohol self-administration (ASA) experiment.
Methods: This was a between-subject double-blind randomized controlled pilot trial. We enrolled 14 non-treatment seeking alcohol dependent (AD) participants who received baclofen 10mg t.i.d. or an 'active' placebo (i.e. cyproheptadine 2mg t.i.d.) for 8 days. Then, participants came to our lab to perform a session consisting of an alcohol cue-reactivity (CR) experiment, followed by an alcohol self-administration (ASA) experiment. Measurements of craving [Alcohol Use Questionnaire (AUQ)], attention [Alcohol Attention Scale (AAS)], salivation, sedation and stimulation [Biphasic Alcohol Effects Scale (BAES)] were performed. Moreover, stress-related hormones were determined, i.e. cortisol, prolactin (PRL) and growth hormone (GH).
Results: 13 out of the 14 participants completed the laboratory session. No severe or serious side effects were reported during the study. There were no significant differences in terms of craving or attention during the CR. As for stimulation and sedation, we found an increase in the baclofen group compared to placebo, stimulation: $F(1,92) = 32.4$, $p < .001$ and for sedation, $F(1,88) = 28.53$, $p < .001$. During the ASA, we found an effect size d of .76, $t(6.4) = 1.43$ with means of 1.43 vs. 0.17 drinks with $p = 0.20$. Furthermore, we found a similar robust effect size $t(12) = 1.20$, $p = 0.25$ of reported drinking during the two days before the ASA. We also found that after controlling for baseline GH, there was a trend toward significance for the baclofen group and lower GH [$r(5) = -0.70$, $p = 0.08$; repeated measures ANCOVAs for GH determined between CR and ASA]. At the end of the ASA, GH levels also correlated positively with the AUQ craving score $r(7) = 0.76$, $p = 0.03$.
Discussion: Although the small sample size, there was a non-significant trend toward statistical significance with a robust effect ($d = 0.76$) in the amount of alcohol consumed during the ASA as well as in reducing standard drinking units (SDUs) consumed on the two days prior to the lab session (after baclofen dose was titrated up). Also, this study design was unique in that this is one of the first studies to conduct the ASA after the CR experiment, resulting in additional alcohol cues other than the priming drink in the ASA. Of particular interest is baclofen's effects on stimulation and sedation during the ASA. It's been suggested that baclofen reduces alcohol drinking by reducing the individual's craving for alcohol. This study shows that the reduction in craving might be secondary to the stimulation and sedation effects from baclofen. Interestingly, sedation was 'controlled' for by using an 'active' placebo that induces sedation. Additionally, the trend toward significance in lower GH levels for baclofen group and the positive correlation between the AUQ scores and GH levels at the end of the ASA suggest a possible link between lower GH and a reduction in alcohol drinking. These several mechanisms may account for the ability of baclofen to reduce alcohol drinking even after individuals have been exposed to alcohol cues and have received an alcohol 'priming' (a model of 'lapse'). Future research is needed to investigate these preliminary findings.
Disclosure: L. Leggio: Part 1: Consultant (ad hoc) for D&APharma. **G. Kenna:** None. **W. Zywiak:** None. **S. Edwards:** None. **S. Fricchione:** None. **T. Tavares:** None. **J. Shoaff:** None. **J. McGeary:** None. **R. Swift:** Part 1: Consultant (ad hoc) for Alkermes and D&APharma, Part 4: Site PI for an Eli Lilly-sponsored multi-site study.

188. Characterization of Operant Intravenous Alcohol Self-administration in Humans: Open-Bar and Progressive-Ratio Paradigms

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Background: Computer-Assisted Self-infusion of Ethanol (CASE) is a method of intravenous (IV) alcohol administration that provides individuals with choices for self-administering alcohol in a laboratory setting, while controlling the breath alcohol concentration (BrAC) using a physiologically-based pharmacokinetic (PBPK) model-based algorithm. The objective of this study was to develop and characterize operant IV alcohol self-administration in non-dependent drinkers using two paradigms: an open-bar paradigm and a progressive-ratio (PR) paradigm.
Methods: Subjects included 64 healthy non-dependent drinkers divided into 2 groups, with each group completing two identical study sessions, between 3 and 30 days apart. Subjects in the first group ($n = 52$) underwent the open-bar self-administration paradigm, which consisted of an initial 25-min priming phase during which the subjects were prompted to push a button to receive individually standardized alcohol infusions, followed by a 125-min "open-bar" phase during which subjects had *ad-lib* access to standardized alcohol infusions, and were instructed to self-administer alcohol to their typical level of exposure in social settings. Subjects in the second group ($n = 12$) underwent the PR self-administration paradigm, which included an initial 25-min priming phase followed by a 125-min "progressive ratio" phase during which subjects had to push the button an increasing number of times for each subsequent infusion. The PR schedule increased exponentially from 10 button-pushes for the first infusion to 54782 for the 20^{th} infusion. During each study session, serial BrAC measurements were obtained. Primary self-administration measures included total number of alcohol rewards (NAR), peak BrAC (PEAK) and average BrAC (AVG). In addition, for the PR method, the total number of button presses (NBP) and average button-press rate (ABPR) were estimated. Subjective measures, obtained repeatedly throughout the study session, included Drug Effects Questionnaire (DEQ) and Alcohol Urge Questionnaire (AUQ). Additionally, recent drinking history (measured using Time-Line Follow-Back), alcohol sensitivity (using the Self-Report of the Effects of Alcohol, SRE), personality (using the NEO personality inventory and Barratt's Impulsivity Scale) were also

assessed to examine their influence on self-administration measures.
Results: There was a high degree of correlation between sessions for all primary self-administration measures for the open-bar (all correlation coefficients > 0.6, $p < 0.001$) as well as for the PR paradigm (all correlation coefficients > 0.81, $p < 0.002$). There was also a high degree of correlation between measures within each session for the open-bar paradigm (NAR vs. PEAK: $R^2 = 0.71$, $p < 0.001$, NAR vs. AVG: $R^2 = 0.79$, $p < 0.001$) and the PR paradigm (NBP vs. PEAK: $R^2 = 0.82$, $p < 0.001$, NBP vs. ABPR: $R^2 = 0.79$, $p < 0.001$), indicating a high level of internal consistency. In the OB paradigm, self-administration measures were significantly associated with drinks per drinking day (NAR: $R^2 = 0.25$; PEAK: $R^2 = 0.31$; AVG: $R^2 = 0.27$, all p values < 0.001), with SRE score, a retrospective measure of alcohol sensitivity (NAR: $R^2 = 0.17$, $p = 0.003$; PEAK: $R^2 = 0.18$, $p = 0.002$; AVG: $R^2 = 0.18$, $p = 0.002$), and with sensitivity to reward (NAR: $R^2 = 0.15$, $p = 0.045$; PEAK: $R^2 = 0.21$, $p = 0.013$; AVG: $R^2 = 0.18$, $p = 0.024$). Preliminary analysis of the PR measures indicated significant association with attentional impulsivity (NAR: $R^2 = 0.44$, $p = 0.007$), as well as with the openness personality facet (NBP: $R^2 = 0.28$, $p = 0.041$). Self-reports of alcohol urges following the priming phase were associated with self-administration measures for both open-bar (NAR: $R^2 = 0.21$, $p = 0.001$) and PR paradigms (NBP: $R^2 = 0.61$, $p = 0.003$).
Discussion: IV alcohol self-administration measures demonstrated substantial test-retest reliability and internal consistency between measures for both open-bar and PR schedules. Open-bar self-administration was significantly associated with recent drinking history as well as alcohol sensitivity, with heavier drinkers and those with low alcohol sensitivity showing higher alcohol self-administration. The PR schedule of self-administration may be related to impulsivity and openness personality traits. Measures of alcohol urges following priming were predictive of both open-bar and PR schedules of alcohol self-administration. The IV self-administration paradigms demonstrate sensitivity to the rewarding effects and motivational properties of alcohol, and are being used to examine the effect of medications being developed for alcoholism pharmacotherapy.
Disclosure: B. Stangl: None. **V. Vatsalya:** None. **M. Zametkin:** None. **D. Hommer:** None. **V. Ramchandani:** None.

189. The Calbindin-D_{28k} Binding Site on Inositol Monophosphatase may allow Inhibition Independent of the Lithium Site of Action

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Background: Among numerous reported biochemical effects the lithium-inhibitable enzyme inositol-monophosphatase (IMPase) remains a viable target for lithium's therapeutic mechanism of action. Calbindin-D_{28}k (calbindin) interacts with IMPase enhancing its activity.
Methods: Modeling the structure of the complex between IMPase and calbindinwas carried out using the program MolFit. The resulting model was used as a basis for designing eleven short linear peptides. Human recombinant calbindin was mutated to exchange residues Asp24 and Asp26 into alanine. Calbindin D24A, D26A was generated by site-directed mutagenesis based on the QuikChange site-directed mutagenesis protocol of Stratagene.IMPase activity in mouse brain homogenates was measured as previously described. Inorganic phosphate liberated from inositol-1-phosphate was quantified spectrophotometrically in an ELISA reader.Liposomes were prepared according to the manufacturer's instructions (Liposome kit: Lipid mixtures for the preparation of liposomes, Sigma.Mice were kept under 12:12 h light/dark cycles in a room with constant temperature (22°C) and food and water *ad libitum*. All experiments were conducted during the light phase of the light/dark cycle. Protocols of the experiments were approved by the Ben-Gurion University of the Negev (Beer-Sheva, Israel) committee for the ethical care and use of animals in research and conducted according to NIH guidelines. Synthesis of cyclic and linear pre-cyclic analog peptides was based on the concept of backbone cyclization.Peptide metabolic stability was measured using rat brush border membrane vesicles.
Results: In the present study *in silico* modeling of IMPase-calbindin binding using the program MolFit indicated that the 55-66 amino acid segment of IMPase anchors calbindin via Lys59 and Lys61 with a glutamate in between (Lys-Glu-Lys motif). The model further suggested that the Lys-Glu-Lys motif interacts with residues Asp24 and Asp26 of calbindin. Indeed, we found that differently from wildtype calbindin, IMPase was not activated by mutated calbindin in which Asp24 and Asp26 were replaced by alanine. Calbindin's effect was significantly reduced by a peptide with the sequence of amino acids 58-63 of IMPase (peptide 1) and by six amino-acid peptides including at least part of the Lys-Glu-Lys motif. The three amino-acid peptide Lys-Glu-Lys or five amino-acid peptides containing this motif were ineffective. Intracerebroventricular administration of peptide 1 resulted in a significant antidepressant-like reduced immobility in the Porsolt forced swim test (FST) compared with mice treated with a scrambled peptide or artificial cerebrospinal fluid. Based on the sequence of peptide 1, and to potentially increase the peptide's stability, cyclic and linear pre-cyclic analog peptides were synthesized. One cyclic and one linear pre-cyclic analog peptides exhibited an inhibitory effect on calbindin-activated brain IMPase activity *in vitro*.
Discussion: These findings may lead to the development of molecules capable of inhibiting IMPase activity at an alternative site than that of lithium. Further studies should investigate whether these peptides modified to exhibit membrane permeability and biological stability or nonpeptide small molecules based on our *in silico* model can mimic behavioral effects of lithium in inositol-related paradigms or other lithium-induced behavioral effects in animals.
Disclosure: O. Almog: Part 1: none, Part 2: none, Part 3: none, Part 4: The study was supported by a NOFAR grant from the Israeli Ministry of Industry, Trade and Labor and by an R&D grant from Johnson & Johnson, Part 5: no. **G. Agam:** Part 1: none, Part 2: none, Part 3: none, Part 4: The study was supported by a NOFAR grant from the Israeli Ministry of Industry, Trade and Labor and by an R&D grant from Johnson & Johnson, Part 5: no.

190. Organic Cation Transporters: Emergence of Novel Therapeutic Targets to Improve Treatment of Depression

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Background: Depression and related disorders are a major public health problem, compounded by the fact that at least half of patients are not effectively treated by currently available medications. Among the most commonly prescribed is the class of selective serotonin (5-HT) reuptake inhibitors (SSRIs), which act to inhibit 5-HT transporter (SERT) mediated 5-HT uptake. The increase in extracellular 5-HT that follows is thought to be critical for initiation of the cascade of downstream events needed for therapeutic effects. Although SERT is the major player regulating high-affinity 5-HT uptake, there is emerging evidence for an important role of organic cation transporters (OCTs) and possibly the plasma membrane monoamine transporter (PMAT) in taking

up 5-HT in brain. This raises the possibility that lack of therapeutic response following SERT blockade could be due to significant 5-HT uptake by OCTs and/or PMAT. Data presented here lend support to this idea.
Methods: High-speed chronoamperometry was used to measure the effect of the OCT/PMAT blocker, decynium-22 (D-22) or the SSRI, fluvoxamine, on clearance of 5-HT from mouse hippocampus, in vivo. The tail suspension test (TST) was used to assay antidepressant-like activity of the same compounds. In vitro, saturation and competition binding assays in hippocampal preparations were used to assess [^{3}H]D-22 binding properties, complimented by quantitative autoradiography of [^{3}H]D-22 binding in coronal sections of brain. Male C57Bl/6 mice were used for all studies.
Results: We previously reported that in SERT-deficient mice, D-22 inhibited 5-HT clearance and produced antidepressant-like effects in the TST whereas D-22 was without effect in wild-type mice (Baganz *et al*., 2008, Proc Nat Acad Sci 105:18976-81). Here we report that pharmacologic blockade of SERT unmasked similar effects of D-22 in wild-type mice; that is, D-22 given in combination with fluvoxamine augmented the 5-HT clearance inhibiting effect of the SSRI as well as its ability to reduce time spent immobile in the TST, an index of antidepressant-like activity. [^{3}H]D-22 binding sites were richly expressed in limbic regions, particularly the hippocampus and cortex, regions important in regulation of mood and overlapping with SERT expression. Competition binding studies showed that [^{3}H]D-22 had no appreciable affinity for SERT, and our preliminary data suggest this is also true for the norepinephrine and dopamine transporters (NET and DAT, respectively).
Discussion: Together, these results reveal a role for OCTs, and/or possibly PMAT, in 5-HT uptake in brain as complementary systems to the high-affinity SERT. Moreover, they emphasize the importance of these low-affinity, high-capacity transporters for 5-HT in regulating the acute action of SSRIs. We previously reported that expression of the OCT3 subtype is increased in SERT deficient mice, whereas expression of OCT1 (Baganz *et al*., 2008, Proc Nat Acad Sci 105:18976-81) or PMAT (unpublished data) did not differ among SERT genotypes. This suggests that OCT3, in particular, may compensate for genetic reductions in SERT expression and/or function. Studies are underway to investigate whether OCT3 responds in a similar fashion to chronic pharmacologic blockade of SERT. If true in humans, then increased expression and activity of OCT3 might account, in part, for poor treatment response in patients with reduced SERT expression, be it genetically or pharmacologically imposed. That said, the current study shows that D-22 can augment the antidepressant-like activity of an SSRI in wild-type mice (i.e. with a full complement of SERT), suggesting that blockade of D-22-sensitive transporters may be especially useful as an add-on treatment in patients who do not respond to SSRIs, regardless of level of SERT expression or activity. OCTs and PMAT are also able to take up norepinephrine, another neurotransmitter important in regulating mood. Indeed, recent studies by Gautron and co-workers (Bacq *et al*., 2011, Mol Psychiatry, e-pub ahead of print) suggest that blockade of D-22 sensitive transporters may also potentiate the antidepressant-like activity of NET blockers and dual SERT-NET blockers, two other commonly prescribed classes of antidepressant drug. Taken together, D-22 sensitive transporters for biogenic amines may account, in part, for poor therapeutic response to current antidepressant medications by limiting the extracellular increase in these neurotransmitters. Our data, together with a rapidly growing literature, support OCTs (and/or PMAT) as legitimate targets for the development of antidepressant drugs with a novel mechanism of action.
Disclosure: L. Daws: None. **D. Apple:** None. **R. Horton:** None. **S. Cano:** None. **M. Vitela:** None. **G. Toney:** None. **G. Gould:** Part 2: None from pharmaceutical or biotechnology companies.. **W. Koek:** None.

191. Role of the5-HT$_7$ReceptorSubtypeinMediating Antidepressant-Like Effects of Lurasidone

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Background: Clinical depression is a common disorder affecting close to 20% of the population. Although effective in many cases, currently available pharmacological treatments remain limited by delayed onset of effect, side effects, and limitedresponse in significant numbersof patients. In recent studies,the serotonin 5-HT$_7$receptor has emerged as a potential new target for the treatment of depression. In animal models it has been shown that blockade or inactivation of this receptor leadsto an antidepressant-like behavioral response. A synergy between currently used antidepressants not acting on the 5-HT$_7$receptor and antagonists of this receptor has also been demonstrated in models of depression. Several atypical antipsychotics have been found to have high affinity for the 5-HT$_7$receptor. Since several members of this class of drug, including amisulpride, have been shown to exert an antidepressant-like effect requiring the 5-HT$_7$receptor, it has thus been suggested that the antidepressant actions of these drugs as either mono-therapy or as augmentation to other drugs is mediated by the 5-HT$_7$receptor. Lurasidone is a newly approved antipsychotic characterized by $D_2$and 5-HT$_{2A}$receptor antagonism, but also a very high affinityantagonist effects atthe 5-HT$_7$receptor. Recently, 5-HT$_7$antagonism has been shown to contribute to the ability of lurasidone as well as amisulpride, to improve novel object recognition in rats treated with subchronic phencyclidine.
Methods: We evaluated the antidepressant-like properties of lurasidone in both acute and chronic animal models of depression. The effects of lurasidone on sleep parameters werealso investigated.
Results: In mice,using the tail suspension test and forced swim testacute models, lurasidone was demonstrated to dose-dependently reduce immobility, an antidepressant-like effect. The effect of lurasidone was absent in mice lacking the 5-HT$_7$receptor, thus demonstrating that this receptor is requiredfor such effects. Furthermore, individually ineffective doses of lurasidone and citalopram when combined also induced an antidepressant-like response in both tests. In a model of depression requiring chronic treatment, the repeated open-space swim model, lurasidone had an antidepressant effect that was similar to citalopram in magnitude. Doses of lurasidone that had antidepressant-like effects did not affect wakefulness, slow-wave sleep, or rapid-eye movement sleep.
Discussion: These results demonstrate that lurasidone in animal models has antidepressant-like properties that are mediated by the 5-HT$_7$receptor. Thus, the data suggest that lurasidone might be suitable for the treatment of depression, either by itself or as augmentation to other therapies. Emerging clinical data suggests the potential for antidepressant effects associated with lurasidone treatment in patients with schizophrenia. The importance of 5-HT$_7$receptor blockade as a target for depression and cognitive impairment is noteworthy.
Disclosure: P. Hedlund: Part 1: Consultant to Dainippon Sumitomo Pharma, Part 4: Dainippon Sumitomo Pharma. **L. Cates:** None. **S. Huitron-Resendiz:** None. **A. Roberts:** None. **H. Meltzer:** Part 1: Consultant to or has receved grant support from Janssen, ACADIA, Bioline, Cypress, Dainippon Sumitomo Pharma, EnVivo, Merck, Otsuka, Pfizer, Roche, and Sunovion. Is a shareholder of ACADIA and SureGene.

192. ERK within the VTA Regulates Adult Behavioral Outputs Induced by Fluoxetine Exposure during Adolescence

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Background: Little is known regarding the mechanisms underlying the neurobiological consequences of antidepressant exposure during adolescence. Here, we assessed for long-lasting effects of adolescent exposure to Fluoxetine (FLX), a selective serotonin reuptake inhibitor, on behavioral reactivity to emotion-eliciting stimuli in adulthood.
Methods: We administered FLX (10 mg/kg, twice daily) to male adolescent C57BL/6 mice (postnatal day 35-49), and assessed their behavioral reactivity to the forced swim test, social defeat procedure, and the elevated plus-maze, 21 days after drug administration. In addition, we examined the role of extracellular signal-regulated kinase (ERK1/2)-signaling within the ventral tegmental area (VTA) in mediating the FLX-induced behaviors using complementary pharmacological and virus-mediated approaches.
Results: Chronic exposure to FLX induced a long-lasting decrease in sensitivity (i.e., stress-resistant) to behavioral despair measures (i.e., social defeat procedure and forced swim test), along with an increased sensitivity to anxiogenic stimuli (i.e., elevated plus maze). Also, FLX exposure during adolescence resulted in decreased ERK2 mRNA within the VTA, 21 days after the last injection. To more directly assess the functional significance of this FLX-induced effect, we increased ERK levels in adult mice pretreated with FLX during adolescence. Here we found that increasing ERK reversed the stress-resistant phenotypes observed after FLX exposure.
Discussion: Treating adolescent c57BL/6 mice with FLX results in enduring complex behavioral outputs indicative of a decreased sensitivity to stress-inducing situations (i.e., a stress-resistant phenotype) in adulthood. This behavioral phenotype is mediated, at least in part, via ERK2 signaling within the VTA. Overall these findings underscore the need for a clearer understanding on the effects of FLX exposure on the developing nervous system.
Disclosure: S. Iñiguez: none. **L. Alcantara:** None. **V. Vialou:** None. **M. Lobo:** None. **E. Nestler:** Part 1: PsychoGenics Merck Neurologix, Part 2: PsychoGenics Merck Neurologix, Part 3: None, Part 4: None. **C. Bolaños-Guzmán :** None.

193. Development of Novel Protein Peptide with Antidepressant-Like Effect

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Background: Dopamine D1 and D2 receptors can form a protein complex with functional properties distinct from those of either D1 or D2 receptors. Although both receptors have been implicated individually in the etiology of mental illnesses, the possibility of a patho-physiological role for the D1-D2 receptor complex remains unexplored.
Methods: We have used co-immunoprecipitation, affinity purification, in vitro binding assay, forced swimming test, learned helplessness test in this project
Results: We have found that the coupling between D1 and D2 receptors was significantly increased in postmortem brain of patients suffering from major depression disorder. Biochemical analyses revealed that D1 and D2 receptors form hetero-dimers via a direct protein-protein interaction between the third intracellular loop of the D2 receptor and the carboxyl tail of the D1 receptor. Administration of an interfering peptide that disrupts the D1-D2 receptor complex significantly reduced immobility in the forced swim test without affecting locomotor activity and robustly decreased escape failures in animals that had developed learned helplessness.
Discussion: Our study provides the first direct evidence implicating the D1-D2 protein complex in the pathology of depression, and also identifies an interfering protein peptide that can disrupt the D1-D2 interaction and exert antidepressant-like effects.Major depressive disorder (MDD) is an illness associated with significant morbidity that may lead to substantial impairment in functioning. With the current clinical antidepressant treatments, only 1/3 of patients achieve full remission of their symptoms after a single trial of antidepressant medications. Even with multiple antidepressant trials, 10-15% of patients continue to experience persistent depressive symptoms and few alternatives have been available for the treatment of resistant symptoms. Thus, alternative novel antidepressant treatments are needed.The identification of D1-D2 interactioninterfering peptide with antidepressant activity may provide a new therapeutic strategy for the treatment of major depression disorder.
Disclosure: F. Liu: None.

194. Effects of Peripherally Restricted Kappa Opioid Receptor Agonists on Pain-Stimulated and Pain-Depressed Behavior in Rats

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Background: Centrally penetrating kappa opioid receptor agonists produce robust antinociceptive effects in most preclinical assays of pain, but they are not effective as analgesics in humans due to limitations in efficacy and/or production of dose-limiting side effects that include psychotomimesis. Kappa opioid receptor agonists that do not readily cross the blood-brain barrier are peripherally restricted after systemic administration and distribute poorly to the central nervous system. Peripherally restricted kappa agonists have promise as candidate analgesics because they may produce antinociception mediated by peripheral kappa receptors more potently than they produce undesirable sedative and psychotomimetic effects mediated by central kappa receptors. This study compared effects of kappa agonists with effects of reference compounds in preclinical assays of pain-stimulated and pain-depressed behavior. Assays of pain-depresssed behavior are valuable in part because (a) they are not vulnerable to "false-positive" effects produced by drugs that produce motor impairment, and (b) they model pain-related depression of behavior and mood.
Methods: Studies were conducted in adult male Sprague-Dawley rats using procedures approved by the VCU IACUC and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Intraperitoneal injection of 1.8% lactic acid (1.0 ml/kg) served as a noxious stimulus to stimulate an abdominal stretching response (a conventional pain-stimulated behavior) and to depress intracranial self-stimulation of the medial forebrain bundle (ICSS; a novel assay of pain-depressed behavior). In the assay of acid-stimulated stretching, antinociception was indicated by a *decrease*in the target behavior. Conversely, in the assay of acid-depressed ICSS, antinociception was indicated by an *increase* in the target behavior. Drug effects on ICSS in the absence of pain were also determined. Effects of six drugs were evaluated: the selective and centrally penetrating kappa agoinst salvinorin A (0.1-3.2 mg/kg), the peripherally restricted kappa agonisttetrapeptide D-Phe-D-Phe-D-Ile-D-Arg-NH2 (a.k.a. ffir; 0.1-10 mg/kg), the nonpeptidic peripherally restricted kappa agonist ICI204448 (3.2-32 mg/kg), the nonsteroidal anti-inflammatory drug and clinically effective analgesic ketoprofen (0.01-1.0 mg/kg), the dopamine uptake

inhibitor and behavioral stimulant cocaine (1.0-10 mg/kg), and the dopamine receptor antagonist and neuroleptic flupenthixol (0.032-1.0 mg/kg). Data were analyzed by one- or two-way ANOVA as appropriate, and significant ANOVA was followed by the Dunnett or Bonferroni post-hoc test.
Results: Only ketoprofen produced significant antinociception in assays of both pain-stimulated and pain-depressed behavior, blocking acid-induced stimulation of stretching and depression of ICSS at a dose that did not alter ICSS in the absence of pain. The centrally penetrating kappa agonist salvinorin A and the dopamine receptor antagonist flupenthixol both significantly decreased acid-stimulated stretching, but also significantly exacerbated acid-induced depression of ICSS and decreased control ICSS in the absence of pain. The peripherally restricted kappa agonists ffir and ICI204448 significantly decreased stretching, but doses that blocked acid-stimulated stretching had little (ICI204448) or no (ffir) effect on acid-induced depression of ICSS, and higher doses tended to exacerbate acid-induced depression of ICSS and decrease ICSS in the absence of pain. Cocaine had no effect on acid-stimulated stretching, but blocked acid-induced depression of ICSS at doses that also stimulated ICSS in the absence of pain.
Discussion: Ketoprofen antinociception in assays of both pain-stimulated and pain-depressed behavior is consistent with the clinical efficacy of ketoprofen as an analgesic. No other drug in this study produced an equivalent antinociceptive profile. In particular, these results do not support the utility of peripherally selective agonists like ffir and ICI204448 for the treatment of pain. Both compounds produced evidence of peripheral selectivity insofar as the reduced acid-stimulated stretching at doses that did not decrease ICSS in the absence of pain. However, despite this apparent selectivity, neither drug was effective in blocking acid-induced depression of ICSS, and high doses of both drugs tended to exacerbate acid-induced depression of ICSS. Salvinorin A and flupenthixol both produced effects consistent with a non-selective behavioral depression, and cocaine produced effects consistent with nonselective behavioral stimulation.
Disclosure: S. Negus: Part 1: Abbott Alkermes Grunenthal Merck, Part 4: Abbott.

195. Reduced Phosphodiesterase-2 Activity in the Amygdala Results in Anxiolytic- and Antidepressant-Like Effects on Behavior in Mice

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Background: Phosphodiesterase-2 (PDE2) is a cyclic nucleotide phosphodiesterase that is highly expressed in the brain and catalyzes the hydrolysis of cyclic AMP and cyclic GMP. Inhibitors of PDE2 such as Bay 60-7550 and ND7001, when administered systemically, produce anxiolytic- and antidepressant-like effects on behavior and reverse the behavioral effects of stress. The behavioral effects of these PDE2 inhibitors are antagonized by ODQ, an inhibitor of soluble guanylyl cyclase, suggesting mediation by cyclic GMP. Experiments were carried using RNAi to verify that reduced PDE2 activity accounts for the behavioral effects observed.
Methods: Male ICR mice were implanted with guide cannula targeting the central nucleus of the amygdala bilaterally. Following recovery from surgery, mice were administered either Bay 60-7550 or lentiviral vector/microRNA targeted to PDE2. The effects of pharmacological inhibition were assessed 30 min post-treatment while those of PDE2 knockdown were assessed beginning one week after treatment with the lentiviral vector/microRNA. Behavioral effects were assessed in the elevated plus-maze and the tail-suspension tests; ODQ was used to assess cyclic GMP involvement. Cannula placement and viral vector localization were determined histologically via its GFP tag.
Results: Administration of Bay 60-7550 into the central nucleus of the amygdala resulted in anxiolytic- and antidepressant-like effects on behavior of mice in the elevated plus-maze and tail-suspension test, respectively; these effects were blocked by pretreatment with ODQ. Viral vector/microRNA-induced knockdown of PDE2 resulted in similar effects on behavior in these tests, which also were blocked by ODQ. The treatment reduced PDE2 expression by approximately 80%.
Discussion: While it is difficult to unambiguously infer the mechanism by which PDE2 inhibitors produce anxiolytic- and antidepressant-like effects on behavior, the present study does provide an additional line of support that reduced PDE2 activity, achieved in this case via lentiviral vector/microRNA-induced knockdown, is associated with such behavioral effects. While PDE2 catalyzes the hydrolysis of both cyclic AMP and cyclic GMP, antagonism of the behavioral effects of both pharmacological inhibition and knockdown by ODQ suggests a predominant role for increased cyclic GMP signaling. This is consistent with previous reports of similar behavioral effects resulting from treatment with NO donors, which also increase cyclic GMP signaling.
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196. Dynorphin and Orexin Interactions in the Development and Expression of Depression-Related Anhedonia

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Background: Both human and animal work suggests that dynorphin neuropeptide function might be increased and orexin peptide function decreased in depression. Since dynorphin and orexin modulate each other's function and play a significant role in reward-related behaviors, an interactive dysfunction between these peptides may induce depression-related anhedonia.
Methods: Male rats [N = 103] were given five 30-minute exposures to social defeat stress, during which rats were placed in the homecage of an aggressive resident rat, or to sham stress in an empty male rats cage. In Study One, rats were given an intraperitoneal (IP) injection with the kappa antagonist nor-binaltorphimine (norBNI), or with saline sixty minutes before defeat. They also received an intracerebroventricular (icv) injection with saline or with the orexinA antagonist SB334867 15 minutes before defeat. This created four IP + icv drug treatment groups: veh + veh, norBNI + veh, veh + SB334867, norBNI + SB334867. Sexual pursuit was then tested 2 and 14-days after defeat to determine whether sexual anhedonia developed in this depression model and whether SB334867 or NorBNI treatment during defeat blocked its *development*. In Study two, rats were not given any drug treatments prior to defeat or sham defeat sessions. A subgroup of these animals was given the sexual pursuit tests. The remaining rats were sacrificed 2-days post defeat and mPFC, VTA, nucleus accumbens and hypothalamus tissue samples were collected for RIA assessment of orexin A, orexin B and dynorphin A expression to determine whether defeated anhedonic animals showed an imbalance in orexin and dynorphin levels within the 3-major regions of the brain reward system or within the hypothalamus.

Results: Social defeat diminished sexual pursuit in both studies and this effect was long-lasting [e.g., study 2: overall test day × defeat group interaction: $F(1,11) = 26.28$, $p < 0.001$). Study One showed that the development of sexual anhedonia was blocked by norBNI or SB334867 pretreatment during social-defeat sessions [veh + veh vs norBNI + veh or vs veh + SB334867 treated rats: p's < 0.05], and that it did so by enhancing orexin release [norBNI + veh vs norBNI + SB23390: $p < 0.05$]. Study Two showed that dynorphin levels remained unaltered in the 3-major regions of the brain reward system in defeated animals, but orexin levels were diminished in the mPFC and VTA [defeat vs sham rats, p's < 0.05]. In the hypothalamus, both orexin and dynorphin were decreased [defeat vs sham rats, p's < 0.05].
Discussion: These findings suggest that dynorphin and orexin stress mechanisms may play an important role in the *development* of anhedonia symptomology, and that they interact in this development. However, anhedonia *expression* may be caused by a stress-induced imbalance in orexin and dynorphin function in mesocortical reward regions, since orexin was diminished here in anhedonic defeated animals while dynorphin remained unaltered. Importantly, nucleus accumbens dynorphin and orexin expression is not implicated in anhedonia expression, since both neuropeptides remained unaltered here. And finally, we show that hypothalamic orexin and dynorphin co-expressing cells may be diminished in depression.
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Disclosure: The authors have no conflicts of interest to disclose.
Disclosure: J. Panksepp: None. **C. Nocjar:** None.

197. 5-HT Regulation of TrkB Signaling: Role of TG2/Rac-1 Pathway

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Background: Serotonin (5-HT) and Brain derived neurotrophic factor (BDNF) are two signaling molecules that play important regulatory roles in the development and plasticity of neural circuits that are known to be altered in psychiatric disorders including depression. The role of BDNF signaling in depressive behavior is substantiated by studies showing decreased levels of BDNF and its receptor, TrkB in postmortem brain as well as peripheral tissues of depressed subjects. In addition, increases in extracellular 5-HT have been shown to enhance BDNF signaling in rodents. However, the mechanism by which 5-HT regulates TrkB signaling is poorly understood. We hypothesize that increased TG2-dependent transamidation of 5-HT to Rac1 inhibits TrkB signaling with depressive phenotype in mice.
Methods: *In vitro* studies were performed in primary cortical neurons. Cells were treated with 5-HT (25 uM) for 24 h and TrkB and Rac1 protein levels were determined by immunoblot analysis. *In vivo* studies were performed in mice with neuronal TG2 overexpression ($TG2^{+/+}$) and WT mice. In mice, the tail suspension test (TST) and forced swim test (FST) were used to evaluate depression-like behaviors, the open field and dark/light box and elevated plus maze tests were used to evaluate anxiety-like behaviors. Schizophrenia-related behaviors (information processing and working memory deficits) were determined in prepulse inhibition, novel arm, and spontaneous alternation tests.
Results: 5-HT treatment for 24 h significantly decreased TrkB and Rac1 protein levels in neurons. $TG2^{+/+}$ mice demonstrated depressive phenotype with reduced Rac1 protein levels in the frontal cortex. We found reductions in TrkB signaling, Hap1 (a huntingtin associated protein involved in intracellular trafficking of endocytic TrkB) levels and its association with TrkB in frontal cortex of $TG2^{+/+}$ mice. Moreover, inhibition of TG2 activity with cystamine attenuated 5-HT-induced Rac1 degradation and association of 5-HT with Rac1, but increased the association of Hap1 and TrkB in cortical neurons.
Discussion: Our data suggest that Rac1 and Hap1 function downstream of TG2 and are involved in linking 5-HT to TrkB signaling. Given the important role of TrkB in neuroplasticity, identifying novel regulatory mechanisms of TrkB by 5-HT may provide avenues to develop newer therapeutics for depression and related psychiatric disorders.
Disclosure: A. Pillai: None. **C. Pandya:** None. **A. Terry, Jr.:** None. **A. Kutiyanawalla:** None.

198. Double Dissociation between the Roles of BDNF in the Antidepressant Effects of Electroconvulsive Treatment and Desipramine

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Background: Forty to fifty percent of the risk for depression is genetic, however the specific genes and their altered expression in relevant brain sites, and their involvement in the effectiveness of current antidepressant treatments, are still poorly understood. Despite this lack of knowledge, some efficient medication and non-medication treatments for depression are available today, but less than 50% of patients show full remission. Obviously, understanding the neuronal mechanisms underlying depression and effective antidepressant treatments, such as electroconvulsive therapy (ECT), is necessary for development of better treatments. Impaired neuronal plasticity and specifically, altered expression of brain-derived neurotrophic factor (BDNF), were shown to play a critical role in depressive behavior and the mechanism of various antidepressant treatments including ECT. Interestingly, opposing roles were suggested for BDNF in the hippocampus and the ventral tegmental area (VTA), while interactions between these regions were shown on various levels. Here, we evaluated whether BDNF plays an essential role in the antidepressant effects of ECT and desipramine, and performed a direct comparison between BDNF manipulations in the VTA and the hippocampus.
Methods: Knockdown (KD) or over-expression (OE) of BDNF was induced in the hippocampus or VTA of rats by localized microinjection of specific lentiviral vectors (LV). The effects of these manipulations on antidepressant outcomes of ECT and desipramine were evaluated in the forced swim test, and BDNF expression levels were measured in reward-related brain regions.
Results: Both ECT and desipramine increased hippocampal BDNF expression, however, hippocampal BDNF KD blocked only the antidepressant effect of desipramine, but not that of ECT. In addition, ECT caused a robust reduction in VTA BDNF levels, regardless to whether rats were or were not subjected to hippocampal BDNF KD. Moreover, VTA BDNF KD alone was sufficient to induce an antidepressant effect. Therefore, we tested whether VTA BDNF OE can alter the antidepressant effect of ECT. Indeed, the major finding of the present study was that VTA BDNF OE completely blocked the antidepressant effect of ECT.
Discussion: We show that the mechanism of desipramine action is dependent on elevation of hippocampal BDNF expression, while the mechanism of ECT action is dependent on reduction of VTA BDNF expression. These findings highlight the differential roles of BDNF in the hippocampus and the VTA with regards to depression and responsiveness to antidepressant manipulations. Therefore, development of novel antidepressant treatments should implement such differential neuroanatomical considerations, and even aim to induce both elevation and reduction of BDNF expression in the hippocampus and VTA, respectively.
Disclosure: D. Taliaz: None. **S. Haramati:** None. **A. Chen:** None. **A. Zangen:** None.

199. Quantitative Proteomic and Metabolomic Profiling of Antidepressant Drug Action Reveals Novel Targets beyond Monoamine Elevation

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Background: Currently used antidepressants elevate monoamine levels in the synaptic cleft. However, it is believed that secondary downstream effects are responsible for alleviating symptoms of depression. We have aimed to identify affected biochemical pathways downstream of monoamine reuptake inhibition by interrogating proteomic and metabolomic profiles in DBA/2Ola mice after chronic paroxetine treatment.
Methods: Quantitative changes in hippocampal protein expression levels were analyzed by in vivo metabolic labeling using ^{15}N-labeled proteins as internal standards for the indirect comparison of paroxetine- vs. vehicle-treated mice. Proteins were separated by SDS gel electrophoresis, in-gel digested and analyzed by LC-ESI-MS/MS. The use of five biological replicates per treatment group facilitated data analysis by uni- and multivariate statistics. Hippocampal and plasma metabolomic changes were investigated using GC-MS profiling in six biological replicates and group differences analyzed by uni- and multivariate statistics.
Results: Multivariate hippocampal data analysis identified 129 protein and 33 metabolite level changes. Combined pathway analysis revealed alterations in cellular processes including synaptic transmission, neurogenesis, neuronal death, protein synthesis, energy metabolism and oxidative stress as well as profound alterations in amino acid metabolism. Pathways affected by antidepressant treatment were related to energy metabolism, amino acid metabolism and hormone signaling. The identified pathways reveal further antidepressant therapeutic action and represent targets for drug development efforts. A comparison of central nervous system to blood plasma metabolite alterations identifiedseveral biomarker candidates for the assessment of antidepressant treatment effects in the periphery.
Discussion: Metabolomic studies hold great promise for the identification of molecular alterations upon drug treatment. To exclude any metabolite level alterations that are caused by environmental factors such as nutritional effects, animals with homogeneous genetic backgrounds and housed under controlled conditions are the preferred study objects. This way inter-individual variability that is commonplace in patient studies can be avoided resulting in a better signal-to-noise ratio of the drug-elicited metabolite level changes. This is the first study identifying metabolite signatures in chronically *paroxetine*-treated DBA/2 mice. We aimed at revealing treatment effects beyond elevation of serotonin levels in the synaptic cleft that are involved in therapeutic antidepressant effects. Understanding the cross-talk between altered metabolomic pathways will greatly enhance our understanding of the drug's mode of action and adverse side effects. Based on the pathway information we revealed putative antidepressant drug targets and biomarker candidates for the assessment of antidepressant treatment effects elicited via novel modes of action. Ultimately, validated biomarkers are meant to be translated into the clinic for a personalized medicine approach.
Disclosure: C. Turck: None. **C. Webhofer:** None.

200. RG1678, a Novel and Potent Glycine Reuptake Inhibitor (GRI), Enhances the Efficacy of Antipsychotics in Animal Models of Schizophrenia

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Background: Deficient signaling through the N-methyl-D-aspartate (NMDA) receptor has been hypothesized to be a key factor underlying symptoms of schizophrenia. Targeting the glycine site of the NMDA receptor has been proposed as an approach to compensate for the reduced receptor signaling. RG1678 is a novel, potent and selective glycine reuptake inhibitor (GRI) presently in clinical phase 3 development for the treatment of negative and sub-optimally controlled positive symptoms in schizophrenia. We report here the in vivo efficacy of RG1678 alone and in combination with antipsychotic drugs in animal models relevant to schizophrenia.
Methods: [^{3}H]L-689,560 (NMDA receptor glycine site antagonist) binding to rat brain membranes, was performed as previously described. The main behavioral outcome measured was locomotor activity in NMRI mice. Graded oral doses of RG1678 (0.1-10 mg/kg), olanzapine (0.01-1 mg/kg), risperidone (0.003-0.45 mg/kg) and clozapine (0.1-3 mg/kg) were administered prior to d-amphetamine (1.5 mg/kg, i.p.) or L-687,414 (50 mg/kg, s.c.), a glycine site antagonist at the NMDA receptor complex. For combination studies, the low doses of RG1678 and antipsychotics were chosen based on their ability to inhibit d-amphetamine and L-687,414. Two types of combination experiments were performed: i) low dose of RG1678 added to graded doses of antipsychotics (L-687,414 and d-amphetamine challenge); ii) low dose antipsychotics added to graded dose of RG1678 (L-687,414 challenge).
Results: RG1678 and the antipsychotics did not bind to the glycine site of the NMDA receptor up to a concentration of 30 íM, as demonstrated by a lack of inhibition of [^{3}H]L-689,560 binding to rat brain membranes. This, therefore, excluded the possibility that their efficacy in the L-687,414 procedure could be ascribed to activity at the glycine site of the NMDA receptor.
L-687,414 and d-amphetamine significantly increased locomotor activity in mice. RG1678 and the antipsychotics prevented the hyperlocomotion induced by L-687,414 and d-amphetamine at doses that had no significant effect on baseline locomotion. In the L-687,414 procedure when low doses of RG1678 were added to risperidone and olanzapine, an effect that was at least additive was observed. Similarly, low dose risperidone and olanzapine added to RG1678 enhanced its efficacy in the same behavioral procedure. In addition, RG1678 also enhanced efficacy of risperidone in preventing amphetamine-induced hyperlocomotion. When RG1678 was combined with clozapine, there was no effect, a minimal effect, or worsening of behavior in L-687,414 and amphetamine procedures.
Discussion: RG1678, a potent GRI, modulates both glutamatergic and dopaminergic tone, via enhancement of NMDA receptor activity. The finding that RG1678 enhanced the effect of antipsychotics suggests that it may be beneficial in ameliorating sub-optimally controlled positive symptoms in schizophrenia.
The lack of effect of RG1678 in combination with clozapine is in line with negative results observed in clinical trials when this antipsychotic was combined with NMDA receptor glycine site modulators.
Disclosure: D. Alberati: Parts 1-5: full-time employee of F.Hoffmann-La Roche. **J. Moreau:** Parts 1-5: full-time employee of F.Hoffmann-La Roche. **R. Mory:** Parts 1-5: full-time employee of F.Hoffmann-La Roche. **J. Wettstein:** Parts 1-5: full-time employee of F.Hoffmann-La Roche.

201. Characterization of the Relationship between Target Occupancy/Modulation and Preclinical/Clinical Responses for the Glycine Transporter 1 (GlyT1) Inhibitor Org 25935

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Background: Selective inhibitors of the glycine transporter I (GlyT1), eg Org 25935, may represent a novel approach for the

effective treatment of schizophrenia. Here we describe the relationship between GlyT1 target occupancy, cerebrospinal fluid (CSF) glycine elevation and activity in preclinical behavioural models for Org 25935. The translation of pre-clinical to human glycinergic signaling was an important consideration in the assessment of compound viability. Org 25935 glycinergic responses as well as proof of reserve capacity (and lack of tachyphylaxis) in healthy male volunteers is also presented. These data play a key role in guiding the selection of dosing regimens for future proof of concept studies.

Methods: Rat GlyT1 occupancy was determined by measuring specific binding of the selective GlyT1 radiotracer, [^{3}H]CPyBP, to whole brain homogenates prepared from male Sprague Dawley rats treated, in-life, with Org 25935. Org 25935 receptor occupancy was measured in Rhesus monkeys by PET imaging with the GlyT1 tracer [^{18}F] MK-6577. Org 25935-mediated changes in glycine levels were determined in rhesus monkeys and rat CSF, and in the medial prefrontal cortex (mPFC) of conscious rats by *in vivo* microdialysis. Conditioned avoidance response (CAR) was determined in rats treated with Org 25935 (1-10 mg/kg ip). The effect of Org 25935 on a prefrontal cortex mediated cognition task was evaluated using an object retrieval task in scopolamine-impaired rhesus monkeys. Blood plasma samples were drawn in all studies in order to measure drug levels. Healthy male volunteers received multiple oral doses of placebo, 4, 8 or 16 mg Org 25935 BID for 13 days and a next higher single dose on day 14 (8, 16 or 32 mg respectively). CSF was collected continuously in fractions of 30 minutes each for 36 hours following the first dose on Day 1 and for 48 hours following the A.M. dose of Day 13 to measure glycine responses and Org 25935. Simultaneously, Org 25935 was measured in plasma at selected time points.

Results: Preclinical receptor occupancy (Rocc) studies estimated plasma Occ_{50} for Org 25935 to be 216 nM (Hill coefficient 1.65) in monkeys and 573 nM (Hill coefficient 1.09) in rats. Significant increases in mPFC glycine levels (rats) and in CSF glycine levels (rats and monkeys) were observed at Org 25935 plasma concentrations corresponding to Rocc of $>$30%. Maximal increases in glycine levels (rat mPFC and rat and monkey CSF) were observed at GlyT1 Rocc 60-70%. In healthy volunteers, the plasma and CSF kinetics of Org 25935 show dose proportionality with a Tmax delay in CSF of about 4 hours. Maximum Org 25935 concentrations in CSF and plasma ranged from 4.06 to 24.3 ng/mL and 127-648 ng/mL,respectively. On Day 1, CSF glycine concentrations increased with a C_{max} observed at about 5-6 hours after dosing. Following the single dose on Day 1, the baseline corrected C_{av} values during the first 12 hours (one dosing interval) were 1.57, 1.73 and 2.05 íg/mL upon 4, 8 and 16 mg Org 25935 respectively. C_{av} during a dosing interval of 12 hours at steady state following the A.M. dose on day 13 and 14 (next higher dose) were 2.12/2.74, 2.46/3.01 and 2.46/3.06 íg/mL respectively. All doses were well tolerated in volunteers. In the rat CAR model, Org 25935 treatment resulted in a modest but significant disruption in conditioned responding at plasma levels corresponding to Rocc$>$ 80%. At these plasma levels, Org 25935 did not produce any escape failures. Scopolamine treatment produced a significant deficit in the accuracy of the object retrieval task measured in monkeys. Reversal of this deficit was observed at doses of Org 25935 (0.1, 0.3 mg/kg po) which achieved GlyT1 Rocc in the range of 15-60%. However, a higher dose (3 mg/kg po, Rocc 98%) Org 25935 failed to significantly reverse the scopolamine deficit.

Discussion: These data show that Org 25935 can effectively elevate glycine levels in the CSF of healthy volunteers and preclinical species. In healthy volunteers, there were no indications for desensitization upon multiple doses. Preclinical behavioral data support the hypothesis that GlyT1 inhibitors may prove to be effective in the treatment of symptom domains associated with schizophrenia. However, these data also suggest a complex relationship between GlyT1 receptor occupancy, CSF glycine changes and behavioral efficacy. As such, careful consideration of GlyT1 receptor occupancies and CSF glycine changes is required in order to guide the selection of dosing regimens for future proof of concept studies in schizophrenia patients.

Disclosure: P. Dogterom: Parts 1-5: Dr Dogterom is a full time employee of MSD The Netherlands. **F. Thomson:** Parts 1-5: Full-time employee of Merck & Co. **R. Hargreaves:** Parts 1-5: Full-time employee of Merck & Co. **T. Hamill:** Parts 1-5: Full-time employee of Merck & Co. **C. Sur:** Parts 1-5: Cyrille Sur is a full-time employee of Merck & Co and is a shareholder of Merck & Co as well. **J. Uslaner:** Parts 1-5: Full-time employee of Merck & Co. **D. Eddins:** Parts 1-5: Full-time employee of merck & Co. **J. Vardigan:** Parts 1-5: Full-time employee of Merck & Co. **S. Jayaraman:** Parts 1-5: Full-time employee of Merck & Co. **J. Morrow:** Parts 1-5: Full-time employee of Merck & Co. **H. Kleijn:** Parts 1-5: Full-time employee of MSD The Netherlands. **J. Schipper:** Parts 1-5: Full-time employee of MSD The Netherlands. **L. Ereshefsky:** Part 1: Employee of California Clinical Trials Med Group Ltd, Part 2: California Clinical Trials Med Group Ltd., Merck Inc., Johnson & Johnson, Teva, Celgene, Part 3: Employee of California Clinical Trials Med Group Ltd, Part 5: California Clinical Trials Medical Group, affiliated with PAREXEL International. **S. Jhee:** Part 1: Full time employee of CCT Med Group Ltd, Part 2: Salary as a full-time employee of CCT Med group Ltd, Part 3: Salary as a full-time employee of CCT Med Group Ltd, Part 5: California Clinical Trials (CCT) Medical Group Ltd. **D. Schoepp:** Parts 1-5: Full-time employee of Merck & Co.

202. Selective TAAR1 Activation Reveals a Novel Approach for Neuropsychiatric Therapy

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Background: Trace amines, the endogenous ligands of the trace amine-associated receptor 1 (TAAR1), also influence the activity of TAAR4, dopamine transporters, dopaminergic, adrenergic as well as serotonergic receptors and therefore selective ligands for the functional elucidation of TAAR1 are needed. We will report on the identification and electrophysiological and behavioral characterization of the first selective and potent TAAR1 agonists. Furthermore, the suitability of TAAR1 as a drug target for various neuropsychiatric indications will be discussed.

Methods: TAAR1 ligands are evaluated for their binding affinity and functional activity at rodent and primate TAAR1 receptors stably expressed in HEK293 cells, for their physicochemical and pharmacokinetic properties, for their effects on the firing frequency of monoaminergic neurons, and for their *in vivo* properties in genetic and pharmacological animal models of CNS disorders.

Results: The TAAR1 agonists are high affinity ligands for TAAR1, have potent functional activity with selectivity over other molecular targets and have good pharmacokinetic properties making these well suited for oral administration. In mouse brain slices, TAAR1 agonists inhibit the firing frequency of dopaminergic and serotonergic neurons in *Taar1* expressing regions, the ventral tegmental area and dorsal raphe nucleus, respectively. Furthermore, modulation of TAAR1 activity alters the desensitization rate of D2 and 5-HT1A receptors as well as the potency of selective D2 and 5-HT1A agonists. *In vivo* TAAR1 activation demonstrates antipsychotic-, anxiolytic- and antidepressant-like activities and

also affects other behavioral dimensions such as attention, cognition and drug-taking.
Discussion: The current results reveal that TAAR1 modulates a variety of neurophysiological functions, presumably via indirect stabilization of monoaminergic neurotransmission. TAAR1 agonists display clear beneficial activity in animal models predictive of neuropsychiatric dysfunctions, fully supporting its therapeutic potential for psychiatric disorders such as psychosis, depression and substance abuse.
Disclosure: F. Revel: Part 5: F. Hoffmann-La Roche. **J. Moreau:** Part 5: F. Hoffmann-La Roche. **R. Gainetdinov:** Part 1: F. Hoffmann-La Roche Ltd., Part 4: Sponsored Research Agreement from F. Hoffmann-La Roche Ltd. **T. Sotnikova:** None. **A. Ferragud:** None. **C. Velazquez-Sanchez:** None. **S. Morairty:** Part 5: SRI International. **T. Kilduff:** Part 5: SRI International. **R. Norcross:** Part 5: F. Hoffmann-La Roche. **A. Bradaia:** Part 5: Neuroservice. **J. Canales:** None. **T. Wallace:** Part 5: SRI International. **M. Caron:** Part 1: Lundbeck, Merck, Forest Laboratories, Omeros Corp, F. Hoffmann-La Roche Ltd., Part 4: Sponsored Research Agreements: Lundbeck A/G USA, Forest Laboratories, F. Hoffmann-La Roche Ltd.; Unrestricted gifts to Duke University (Caron Lab): Lundbeck A/G. **J. Wettstein:** Part 5: F. Hoffmann-La Roche. **M. Hoener:** Part 5: F. Hoffmann-La Roche.

203. Subchronic Treatment with Lurasidone has both P*reventive* and E*nduring Reversal* Effects on the Phencyclidine (PCP)-Induced Deficit in Novel Object Recognition (NOR) in Rats

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Background: Hypoglutamatergic cortical function is believed to contribute to cognitive deficits in schizophrenia. Subchronic treatment of rodents with the NMDA receptor non-competitive antagonist, phencyclidine (PCP), has been postulated to model this deficit, e.g. by inducing prolonged deficits in novel object recognition (NOR), which may be analogous to deficits in declarative memory in schizophrenia.We have reported that acute treatment with atypical antipsychotic drugs (APDs) including lurasidone, a 5-HT_{2A}/D_2 antagonist and 5-HT_{1A} partial agonist, or tandospirone, a 5-HT_{1A} partial agonist, but not typical APDs (e.g. haloperidol) or pimavanserin, a selective 5-HT_{2A} inverse agonist, reverses the subchronic PCP-induced deficit in NOR (Snigdha *et al.* 2010; Horiguchi *et al.* 2011). We have now tested the *preventive* effect of these drugs on the subchronic PCP-induced NOR deficit. We also tested the hypothesis that subsequent subchronic treatment with lurasidone or tandospirone would produce the *enduring reversal* effect of this deficit.
Methods: Female Long-Evans rats received vehicle or PCP (2mg/kg, b.i.d.) for 7 days(day 1-7), followed by a 7-day washout period (day 8-14; n = 6-9per group). For the *prevention* study, the rats were also administrated lurasidone (1mg/kg), tandospirone (5mg/kg), haloperidol (1mg/kg) or pimavanserin (3mg/kg) b.i.d. 30min prior to receiving each injection of PCP. NOR was tested in the naïve- or PCP-treated rats on day 15 (lurasidone-treated rats were also tested on day 22). For the *enduring reversal* study, the rats were administrated lurasidone (1mg/kg) or tandospirone (5mg/kg) for 7 days after the washout period (day 15-21) and NOR was tested on day 22, 29 and 36. All drugs were administrated intraperitoneally. The procedure has been described elsewhere (Snigdha *et al.* 2010).
Results: Subchronic treatment with PCP induced an enduring NOR deficit, as predicted. In the *prevention* paradigm, administration of lurasidone or tandospirone, but not haloperidol or pimavanserin, prior to PCP, significantly prevented the PCP-induced NOR deficit when tested on day 15 or 22 (only lurasidone was tested on day 22). In the *enduring reversal* paradigm, subsequent subchronic treatment with lurasidone or tandospirone significantly reversed the NOR deficit induced by PCP on day 22. The ameliorating effect of lurasidone was seen on day 29 and day 36. On the other hand, tandospirone showed only partial amelioration on day 29 and no effect on day 36.
Discussion: This is the first report of the ability of atypical APD (lurasidone), or a 5-HT_{1A} agonist (tandospirone) to prevent the effect of subchronic PCP-induced disruption in rat NOR. Haloperidol and pimavanserin were ineffective, as they are in acute reversal studies (Snigdha *et al.* 2010). These results suggest that lurasidone, the atypical APD, or tandospirone, a 5-HT_{1A} agonist, might be able to partially or completely prevent the development of some domains of cognitive impairment in individuals at risk for schizophrenia or related disorders, with similar cognitive impairment, e.g. bipolar disorder. This is also the first report which showed the long-lasting (2 weeks) ameliorating effect of subsequent subchronic treatment with an atypical APD (lurasidone) on subchronic PCP-induced NOR deficit in rats, at least when administered shortly after the PCP treatment. Subchronic treatment with tandospirone, a 5-HT_{1A} agonist, had less persistent ability to ameliorate the effect of PCP. These results are consistent with the previous studies which showed the subsequent treatment with some atypical APDs reverse the NOR deficit induced by PCP in mice (Hagiwara *et al.* 2008; Nagai *et al.* 2009). It is noteworthy that the effect of lurasidone, a multireceptor-acting drug, was more enduring than that of tandospirone, a highly selective drug. These results suggest that 5-HT_{1A} agonism is a more effective means of reversing the effect of subchronic PCP on NOR than is 5-HT_{2A} inverse agonism or D_2 antagonsim, but that the combination of all three actions in one drug, plus 5-HT_7 antagonism (lurasidone) is even more effective. There is little data as of yet to suggest that these results with *prevention* and *enduring reversal* of the effect of subchronic PCP translate into the clinical arena. Specific studies to test whether or not they do translate would be of great interest to test the limits of the hypoglutamate animal model of human psychotic disorders.
Disclosure: M. Horiguchi: Part 5: Dainippon Sumitomo Pharma Co., Ltd. **K. Hannaway:** None. **A. Adelekun:** None. **H. Meltzer:** Part 1: Acadia Dainippon Sumitomo Eli Lilly Envivo Janssen Neurotherapeutics Pfizer Roche Sunovion Suregene, Part 2: No, Part 3: Consulting, Part 4: Dainippon Sumitomo, Part 5: No.

204. Relationship between In Vivo Receptor Occupancy and Efficacy of Novel Metabotropic Glutamate Receptor Subtype 5 Allosteric Modulators with Different in Vitro Profiles of Activity at $mGlu_5$

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Background: Preclinical and clinical studies suggest that selective activation of the metabotropic glutamate receptor subtype 5 ($mGlu_5$) has exciting potential for development of novel therapeutic strategies for treatment of schizophrenia. This hypothesis is based on evidence that $mGlu_5$ and N-methyl-D-aspartate (NMDA) glutamate receptors are closely associated signaling partners in forebrain circuits and that enhanced signaling through the NMDAR may have antipsychotic activity. A lack of success in developing highly subtype-selective agonists that act at the orthosteric glutamate binding site and exhibit adequate properties for in vivo studies has led to the development of a novel approach to the activation of $mGlu_5$ using highly selective positive allosteric

modulators (PAMs) of this receptor. As opposed to direct activation of $mGlu_5$, PAMs dramatically potentiate the response of the receptor to its endogenous ligand glutamate and offer high selectivity for the targeted receptor. Consistent with this, highly selective PAMs of $mGlu_5$ have robust effects in animal models used to predict efficacy of novel antipsychotic agents. In addition, $mGlu_5$ PAMs enhance multiple forms of synaptic plasticity in the CNS and have cognition-enhancing effects in animal models. Studies within our laboratory suggest that PAMs of $mGlu_5$ may provide a novel approach for the treatment of the psychotic symptoms and cognitive impairments observed in individuals with schizophrenia.

Methods: The in vivo efficacy of a potent, selective $mGlu_5$ PAM was assessed in reversal of amphetamine-induced hyperlocomotion, a rodent model predictive of antipsychotic activity.

Data were analyzed by a one-way analysis of variance with comparison with the vehicle + amphetamine control group using Dunnett's test. Calculations were performed using JMP 8 (SAS Institute Inc.) statistical software. Molecular characterization of the novel compound was conducted in calcium mobilization and inhibition binding assays using HEK cells overexpressing the $mGlu_5$ receptor. In addition, positron emission tomography (PET) imaging was used to determine receptor occupancy of a novel $mGlu_5$ PAM in discrete regions of the rat brain utilizing the highly selective radioligand, [^{18}F]FPEB. These in vivo microPET imaging techniques were used to determine a dose-response relationship for receptor occupancy at the allosteric MPEP site of $mGlu_5$. The data for the dose-response studies were analyzed using a between-group analysis of variance. If a main effect of dose was determined, then each dose group was compared with the vehicle control group using a Dunnett's comparison.

Results: The selective $mGlu_5$ PAM caused a significant, dose-dependent reversal of amphetamine-induced hyperlocomotion, indicative of its antipsychotic activity. In depth molecular characterization of this compound suggests a fully competitive interaction with the allosteric MPEP site of $mGlu_5$. These data are further supported by microPET imaging studies demonstrating a dose-dependent displacement of the highly selective MPEP-site radioligand, [^{18}F]FPEB. In addition, in vivo receptor occupancy of the allosteric MPEP site bears a close relationship with in vivo efficacy in preclinical models indicative of antipsychotic activity for this compound.

Discussion: PET radiotracers are valuable preclinical tools to evaluate receptor occupancy and guide the dosing regimen of potential therapeutic drugs. Interestingly, these data demonstrate that relatively low occupancy of the allosteric site of $mGlu_5$ is required to achieve maximum in vivo efficacy. The dose-dependent reversal of amphetamine-induced hyperlocomotion studies demonstrate that the minimum dose that evokes maximum efficacy produces receptor occupancy of only 32%. These data support previous in vitro findings published in this laboratory depicting that high cooperativity between $mGlu_5$ PAMs' affinity and potency/efficacy exists. Particularly exciting is the close correlation between in vivo receptor occupancy and efficacy for this compound. These studies provide valuable insights that are currently guiding lead optimization efforts aimed at advancing novel $mGlu_5$ PAMs into clinical development.

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205. High-Throughput Screening for Allosteric Modulators of the D_2 Dopamine Receptor

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Background: The D_2 dopamine receptor (DAR) is central in the etiology and/or therapy of many neuropsychiatric disorders. Specifically, D_2 DAR antagonism is the hallmark of all FDA-approved antipsychotics and stimulation of the D_2 DAR is critical for effective antiparkinsonian therapy. Unfortunately, truly specific drugs for this receptor have been difficult to obtain, primarily due to high conservation of the orthosteric binding site (where dopamine binds) within DAR subtypes and between other G protein-coupled receptors. Another approach to receptor-selective ligands is to identify allosteric modulators that bind to less conserved sites on the receptor and have the potential to be exquisitely selective. Such allosteric modulators can exhibit a variety of functional activities including positive or negative modulation of ligand interactions at the orthosteric site, modulation of agonist efficacy, including engendering functional selectivity, or they can exhibit agonist efficacy of their own. In order to identify allosteric modulators of the D_2 DAR, we have developed a high throughput-screening (HTS) platform to interrogate large chemical compound libraries. Our goal is to identify novel small molecule scaffolds with the desired functional characteristics and define their mechanisms of action and selectivities among the DAR subtypes.

Methods: The primary HTS assay utilizes a cell line expressing the D_2 DAR coupled to a chimeric Gqi5 protein, thereby linking receptor activation to robust Ca^{2+} mobilization that is measured using a fluorescent readout. We have also developed HTS-formatted secondary assays to measure orthogonal D_2 DAR activities (cAMP modulation or beta-arrestin interactions) as well as HTS-based counter-screening assays to determine selectivities between other DAR subtypes (D_1, D_3, D_4 and D_5). Interaction of hit ligands with the orthosteric binding site of the D_2 DAR was assessed using standard radioligand binding competition assays.

Results: Through the NIH Molecular Libraries Program, a 370,000 + small molecule library was screened to identify agonists (allosteric or orthosteric), positive allosteric modulators, or antagonists (allosteric or orthosteric). From this primary screen, 2,288 compounds with agonist activity, 1,408 compounds with potentiator activity, and 2,294 compounds with antagonist activity were cherry-picked to maximize the chances of identifying chemical series with unique activities. Upon further evaluation, none of the potentiators confirmed when retested while 650 agonists and 858 antagonists did not confirm. The remaining confirmed agonist and antagonist ligands were subjected to orthogonal and counter-screening functional assays. On the basis of these analyses, 745 agonist and 499 antagonist compounds were evaluated using radioligand competition binding assays as a filter to separate orthosteric and allosteric ligands. Any compound that competes for radioligand binding to the orthosteric site of the receptor is likely to be orthosteric in nature, while any compound that is ineffective in competing for binding is likely to exert its functional activity via an allosteric mechanism. These experiments resulted in the identification of 47 agonists and 48 antagonists that had insignificant effects on radioligand binding when tested using up to 40 uM of library compound. Many of these compounds exhibited their maximal functional effects (either agonist or antagonist activities) at concentrations that had no effect on radioligand binding to the orthosteric site. These compounds would thus appear to be allosteric agonists and negative allosteric modulators of the D_2 DAR.

Discussion: We have conducted a high through-put screen of the NIH Molecular Libraries Program small molecule repository

containing nearly 400,000 compounds. Our goal for this screen was to identify compounds with agonist, antagonist, or potentiator activity at the D_2 DAR. We were specifically interested in discovering ligands that exert their functional effects via allosteric mechanisms. However, a by-product of this screen is also the identification of compounds that, while orthosteric in nature, may exhibit high selectivity for the D_2 DAR and/or are functionally selective with respect to D_2 DAR signaling pathways. Unfortunately, this screen did not yield positive allosteric modulators that potentiated dopamine's action at the D_2 DAR. However, numerous lead compounds with agonist or antagonist activities were identified that appear to exert their functional effects via allosteric mechanisms. The most promising of these ligands are undergoing further characterization.

Disclosure: D. Sibley: None. **R. Free:** None. **N. Southall:** None. **T. Doyle:** None. **R. Roof:** None. **J. Conroy:** None. **Y. Han:** None. **J. Javitch:** None. **M. Ferrer:** None.

206. The Novel Opioid Receptor Modulator RDC-0313 (ALKS 33) reduces Olanzapine-Induced Weight Gain and Adipose Accretion in a Novel nonhuman Primate Model of Antipsychotic-Related Weight Changes

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Background: Antipsychotics (ATAP) can cause metabolic dysfunction and weight gain. Female patients have a 3.6-fold increased risk of weight gain than male patients (Hakko *et al.*, 2006) and increases in BMI can be observed in as little as 1 week post initiation of treatment (Kluge et. al., 2009). Weight gain has been reported with risperidone, clozapine, sertraline and olanzapine (OLZ).

ALKS 33 is a new chemical entity, currently under development for central nervous system-related disorders that acts as an antagonist at μ opioid receptors, with mixed agonist/antagonist activity at κ and δ receptors. We have previously demonstrated in rats that co-administration with ALKS 33 mitigates OLZ-induced weight gain whereas naltrexone does not (SFN, 2010). We are unaware of studies using nonhuman primates (NHP) to investigate OLZ-induced changes in weight gain or metabolic effects. Consequently, we investigated if: 1) OLZ would cause similar metabolic changes in female cynomolgus monkeys as seen in humans; and 2) would ALKS 33 attenuate OLZ-induced changes.

Methods: Three groups of late-adolescent female cynomolgus monkeys ($n = 5$/group; 4.04 ± 0.05 yrs) were used for this study: 1) vehicle control; 2) OLZ only; and 3) OLZ with ALKS 33. Beginning two weeks prior to the study, monkeys were given ad libitum access to a highly palatable, high caloric diet. On the day prior to the start of treatment, monkeys were weighed and assigned to groups using a randomized block design based on body weight (BW); average BW across the 3 groups was 3.1 ± 0.08 kg on the day of randomization. Also, baseline whole body CT scans were taken on the day prior to initiation of treatments. Monkeys receiving OLZ were dosed twice daily (6 hours between doses) for 28 days. The initial daily dose of OLZ was 1 mg/kg (PO, in 1% methylcellulose) and increased every 3 days to a daily dose of 6 mg/kg by Day 10. For group 3, ALKS 33 (0.4 mg/kg, IM) was administered in the morning immediately after administration of OLZ. Weights were taken every 3 days and on Day 28 blood samples were collected for serum chemistry analysis. A second CT scan was conducted on day 29.

Results: BWs were relatively constant for two months prior to the initiation of ad libitum feeding. Vehicle treated monkeys gained an average of 0.28 kg (9% of Day 0 BW) over the 28-day study. This gain was attributed to the ad libitum feeding of the highly palatable diet. Over the same 28-day period, OLZ-treated monkeys gained an average of 0.46 kg (15% of Day 0 BW). This marked increase in average weight gain was driven by 3 of the 5 monkeys who gained between 19.8 and 37.8 % of their initial body weight. An accretion of adipose tissue was observed in all monkeys compared to baseline control values. However, monkeys in the OLZ group gained relatively more adipose tissue compared to the vehicle group. Also, there was a difference in the location of adipose tissue deposition with the OLZ group showing more abdominal fat accretion. Concentrations of triglycerides (TGs) and LDL were higher in OLZ-treated monkeys (86.6 and 105.8 mg/dL, respectively) compared to the vehicle group (62 and 87.8 mg/dL, respectively). In monkeys treated with OLZ and ALKS 33 the average BW gain over the 28-days was only 0.08 kg (2.6% of Day 0 BW). While these monkeys also gained adipose tissue, the extent and distribution of fat was similar to that observed in the vehicle group and lower than the OLZ-only group. Finally, co-administration of ALKS 33 prevented OLZ-induced elevations in TGs and LDL concentrations.

Discussion: Based on these data, treatment of nonhuman primates with OLZ for 28-days resulted in qualitative changes in weight, adipose tissue accretion, TGs and LDLs similar to those reported in patients. Furthermore, OLZ-induced changes were mitigated by co-administration of ALKS 33.

Disclosure: M. Todtenkopf: Part 5: Alkermes, Inc. **R. Dean:** Part 5: Alkermes, Inc. **M. Brunner:** Part 5: Battelle. **M. Knopp:** None. **D. Deaver:** Part 5: Alkermes, Inc.

207. Effects of Oxytocin on Social Symptoms in Adults with Schizophrenia: Cognition and Physiology

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Background: Patients with schizophrenia (SCHZ) have social cognitive deficits, and experience difficulty recognizing facial emotion, interpreting paralinguistic cues (e.g., sarcasm) and understanding others' mental states (theory of mind). These deficits impair quality of life and are resistant to available treatments. Patients also have olfactory deficits and decreased parasympathetic (PNS) activity, which are both associated with worse negative symptoms. The neuropeptide oxytocin (OT) has been implicated in bonding, has powerful pro-social effects when administered to humans, and has shown promise in enhancing social cognitive functioning in SCHZ. Further, OT signaling has been implicated in socially relevant olfaction in animals and intranasal OT increases PNS activity in healthy subjects (HS). Therefore, we investigated the effects of intranasal OT administration on social cognition, olfaction and PNS activity in patients with SCHZ and HS.

Methods: We administered OT (40IU) and placebo (PL) intranasally to 22 male adult patients with SCHZ and 8 HS of similar age and educational level in a randomized, double-blind, cross-over, within-subject study, with the two days of testing separated by one week. We measured performance on The Awareness of Social Inference Test (TASIT), which uses short video clips of actors to assess subjects' ability to comprehend counterfactual statements from paralinguistic cues signaling sarcasm (*Sarcasm Items*) and to make judgments about the actors' thoughts (*Theory of Mind Items)*. We also measured performance on Reading the Mind in the Eyes Test (RMET), which tests facial emotion recognition. Olfactory thresholds were measured for Lyral, Clove, and Anise oils using a modified Munich Olfaction Test. Subjects identified the bottle with the testing compound from amongst two mineral oil containing distractor bottles in an upward step procedure with increasing geometric dilutions (12.5×10^{-6}% to 20% m^3/m^3). Respiratory Sinus Arrhythmia (RSA), an index of PNS activity,

was assessed from electrocardiogram data as the natural log of heart rate variance across the respiratory band (.12-.4 Hz). Paired t-tests were used for all comparisons and data is expressed as Mean ± (S.E).

Results: OT administration to SCHZ patients (Age:43.1 (9.4), Education: 13.5 (1.7)) improved performance on *Sarcasm Items* (70% ± 3% vs. 64% ± 4%, $p < 0.05$) and *Theory of Mind Items* (74% ± 4% vs. 64% ± 5%, $p < 0.03$) while OT administration to HS (Age: 42.6 (14.2), Education: 15.9 (2.1)) impaired performance on *Sarcasm Items* (80% ± 4% vs. 89% ± 3%, $p < 0.03$) and had no effect on *Theory of Mind Items* ($p = 0.4$). In SCHZ subjects, OT led to trend-level improvement in RMET performance (68% ± 3% vs. 65% ± 3%, $p < 0.1$) and enhanced detection of Lyral (but not Anise or Clove) at lower concentrations (4×10^{-3}% ± 4×10^{-5}% m^3/m^3 vs. 2×10^{-2}% ± 5×10^{-5}% m^3/m^3, $p < 0.1$). A subsample of SCHZ patients ($N = 4$) showed non-significantly lower RSA at baseline than HS ($N = 4$, 5.2 ± 0.6 vs. 5.6 ± 0.6), and OT increased RSA in these patients 75 minutes after administration (change from baseline: 1.7 ± 1.6 vs. -0.2 ± 0.6, $p = 0.22$).

Discussion: Our preliminary findings indicate that a single dose of OT: 1) Significantly improved patients' ability to understand others' mental states and comprehend sarcasm, and improved at trend level patients' ability to recognize facial affect; this suggests that oxytocin remediates the social cognitive deficits in SCHZ and is clinically promising because these abilities have all been correlated with better functional outcomes in SCHZ. 2) Improved olfactory performance (at trend level) on detecting Lyral, which suggests that, A) OT might be the first pharmacological agent to remediate olfactory deficits in SCHZ because patients and their unaffected family members have specific impairments in detecting Lyral by smell, and B) this olfactory improvement may have social consequences because difficulty smelling Lyral is correlated with worse negative symptoms and decreased social motivation in SCHZ. 3) Increased RSA (non-significantly), a measure that is known to reflect PNS activity, is correlated with enhanced social cognition and engagement and decreased mortality in HS and is known to be pathologically low in SCHZ; suggesting that OT may remediate this autonomic dysfunction and lead to decreased mortality and morbidity in SCHZ. In sum, our initial data provide preliminary support for investigating OT as adjunctive pharmacological treatment for social cognitive and physiologic deficits in SCHZ. Larger studies are needed to confirm and extend our findings.

Disclosure: J. Woolley: None. **B. Chuang:** None. **J. Resa:** None. **K. Rankin:** None. **S. Vinogradov:** Part 1: Sophia Vinogradov is a consultant to Brain Plasticity Institute, a company with a financial interest in cognitive training software.

208. Hard-Wired for Hedonism: The Role of Cortical D1 Dopamine Receptors in Reward Processing

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Background: Motivational salience is important for associating environmental stimuli with action. During addiction, motivational salience plays an important role in establishing cues associated with drugs of abuse with their rewarding properties. The D1 dopamine receptor on glutamate neurons has previously been linked with motivational salience and reinstatement to drug cues. However, we hypothesized that this D1 receptor may play an important role in establishing the *initial* reward-cue link rather than a consequence of repeated drug exposure. Through the use of genetically-engineered lentiviral vectors, we demonstrate that over-expression of D1 receptors on glutamate neurons in the prelimbic prefrontal cortex increases vulnerability to seek out or consume rewarding substances.

Methods: Sprague-Dawley adult male rats were transduced with a lentiviral vector that was specific for glutamate neurons (e.g., via the CamKIIa propmotor). Vectors expressed either D1 or control GFP (CK.D1 or CK.GFP) stereotaxically injected into the prelimbic prefrontal cortex (plPFC). Five-six days later, rats were tested for place preferences to environments associated with 10 mg/kg cocaine, 0.6 mg/kg nicotine, 0.5 g/kg alcohol, or 125 mEqv lithium chloride. Each of these doses was selected as they show minimal preferences or an aversion to their associated environments in adult rats. In order to specify that D1 effects were localized to glutamate neurons, a separate group of rats were transduced with a vector that expressed D1 in all neurons (via the synapsin promotor; syn.D1) and tested for cocaine-associated place preferences. Additionally, we were interested in determining whether elevated D1 on glutamatergic neurons would increase preference for other reward-related stimuli; a two-bottle sucrose preference test was conducted to 0.25, 0.5, and 1% sucrose solutions.

Results: Genetically–engineered increases in D1 on glutamate neurons in the plPFC, but not CK.GFP, increased the preference for cocaine- and nicotine-associated environments, and decreased the aversion to the alcohol-associated environment (virus × conditioning interactions: cocaine: $F_{1,11} = 12.7$, $P = 0.004$; alcohol: $F_{1,8} = 5.9$, $P = 0.046$; nicotine: $F_{1,9} = 10.9$, $P = 0.009$). Significant differences were observed between virus conditions in the post-conditioning effects: cocaine: $P = 0.001$; alcohol: $P = 0.02$; and nicotine: $P = 0.047$. Lithium chloride produced a post-conditioning aversion in all conditions relative to pre-conditioning values ($F_{1,12} = 17.4$, $P = 0.001$), and CK.D1 and CK.GFP subjects did not differ in this aversion ($P = 0.87$). Heightened place preferences for cocaine required D1 over-expression on glutamatergic neurons exclusively, since nonspecific syn.D1 had no effect on cocaine place conditioning ($P = 0.35$). When tested for consumption of sucrose solutions, a leftward shift was observed in the concentration-curve of sucrose, with CK.D1 animals consuming more sucrose solutions than CK.GFP animals. In addition, when given access to this high caloric substance, CK.D1 subjects further demonstrated a steeper slope of weight gain ($r = 5.31$) compared with CK.GFP ($r = 2.85$) and control subjects ($r = 2.39$).

Discussion: These data suggest that overexpression of D1 dopamine receptors on glutamatergic prefrontal cortex neurons plays a vital role in establishing vulnerability to rewarding substances (cocaine, nicotine, alcohol, and sucrose). In contrast, CK.D1 does not increase the motivational salience for aversive qualities, as a reduction in the aversion to environments associated with lithium chloride did not differ from CK.GFP controls. Our data raise the possibility that D1 signaling on glutamate neurons is a significant risk factor for addiction and other reward-related disorders, and not merely a consequence of drug exposure.

Disclosure: H. Brenhouse: Part 1: Heather Brenhouse had previous financial involvement with Infinity Pharmaceuticals that is unrelated to the research presented here. **N. Freund:** None. **B. Thompson:** None. **K. Sonntag:** None. **S. Andersen:** None.

209. A role for Noradrenergic Systems in Kappa-Opioid Dependent Stress and Drug Seeking Behaviors

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Background: Prior reports have demonstrated that stress causes dynorphin release, activating kappa-opioid receptors (KOR) in monoamine circuits (dopamine/serotonin). This activity results in dysphoria-like behavioral responses, potentiation of cocaine conditioned place preference and reinstatement of drug seeking. Additionally, it has been reported that stress-induced reinstatement behavior and withdrawal-induced aversion (dysphoria) are mediated by activation of pre- and post-synaptic noradrenergic

receptor systems. The primary sources of forebrain norepinephrine are the pontine locus coeruleus (LC) and the brainstem A1/A2 nuclei. Together, these reports suggest a possible link between the noradrenergic and dynorphin/kappa opioid circuitry. The anatomical and behavioral implications of this putative interaction have not been demonstrated.

Methods: We investigated the role of noradrenergic circuits in KOR-dependent conditioned place aversion (CPA) and stress-induced reinstatement of drug seeking using conditioned place preference models (CPP). The CPA paradigm measured KOR-agonist U50488-mediated aversion in the presence and absence of the â-adrenergic receptor antagonist propranolol in male C57BL/6 mice. The CPP paradigm measured stress-induced reinstatement of cocaine preference. We also investigated the anatomical relationship between KOR, noradrenergic and GABAergic cells in the LC using immunohistochemical approaches and site directed KOR-inactivation with the selective KOR-antagonist norBNI.

Results: As previously reported by several groups we also show here that KOR activation causes reinstatement of cocaine place preference. KOR-induced reinstatement of drug seeking is thought to be driven by the dysphoria-like properties associated with KOR activation. Using a CPA model we found that injection of propranolol (10mg/kg) prior to injection of the KOR-agonist U50,488 (2.5mg/kg), blocked KOR-dependent CPA. To determine if â-adrenergic receptor blockade also prevents KOR-induced reinstatement of CPP, mice were pre-treated with propranolol (10mg/kg), followed by the injection of the KOR-agonist U50,488 (5.0mg/kg), and then exposed to the final post test. Unexpectedly, â-adrenergic receptor antagonism concomitantly with KOR activation did not block reinstatement of CPP, but instead significantly potentiated ($p < 0.05$) reinstated cocaine place preference. This effect was dependent on KOR activation as â-adrenergic blockade alone did not cause reinstatement. Recent data suggests that dynorphin peptide products are located within the LC, the primary source of norepinephrine in the forebrain. Consistent with this finding, we report that phospho-KOR-ir (activated KOR) is expressed in the LC following KOR agonist treatment (U50488,10mg/kg, i.p., 30 min). In order to further dissect the neural circuitry of KOR-mediated â-adrenergic dependent potentiation of CPP reinstatement we used local injection of KOR antagonist, â-antagonist, and optogenetic approaches to investigate the role of targeted LC stimulation and KOR blockade on CPA and reinstatement.

Discussion: These data suggest that reinstatement of cocaine preference may be modulated by KOR-dependent disinhibition of noradrenergic systems that typically suppress drug reward output circuitry; however this hypothesis requires further exploration. Together, these data suggest that there is a causal connection between the dynorphin/KOR circuit and noradrenergic systems in aversion and cocaine preference behaviors. Supported by NIDA, R00DA025182 (MRB)

Disclosure: R. Al-Hasani: None. **J. McCall:** None. **M. Bruchas:** Part 1: None, Part 2: None, Part 3: None, Part 4: NIH, 2009-Present, National Institute on Drug Abuse, Part 5: NA.

210. The Effects of Small Molecules on Trace Amine-Associated Receptor 1 (TAAR1) Inform on its Contribution to Methamphetamine Abuse

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Background: Trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor that has been detected in numerous brain regions and various tissues of multiple species including humans and rodents. Notably, TAAR1 is present in the dopamine-rich ventral tegmental area (VTA) of the midbrain, a region involved in the expression of motivated behavior. When heterologously expressed in cell culture TAAR1 stimulates the production of cAMP when exposed to the noncatecholic biogenic trace amines beta-phenylethylamine (PEA), para-tyramine, octopamine and synephrine. Interestingly, TAAR1 is also activated by amphetamine, methamphetamine (METH) and 3-iodothyronamine, a close relative of thyroid hormone. Though its biology is largely unknown TAAR1-mediated signaling has been implicated in cognitive disorders, including addiction. Based on its *in vitro* pharmacological profile we hypothesize TAAR1-mediated signaling contributes to the psychostimulant effects and addictive properties of METH.

Methods: We are taking a multidisciplinary approach to investigate the role of TAAR1 in METH abuse. First, we are designing and synthesizing novel low molecular weight compounds that we then use in an *in vitro* cell culture based functional (cAMP) assay of TAAR1 activation to define the structure-activity profiles of these proprietary compounds. Those chemical entities whose synthesis is straightforward and *in vitro* profile is promising (e.g. antagonists of PEA-stimulated cAMP production) are then evaluated for their effects on METH-induced open field activity and intravenous METH self-administration in wild type and Taar1-deficient adult male mice.

Results: We have identified a compound that when administered intraperitoneally to adult male wild type C57Bl/6 mice is ~100 times more potent and efficacious than the Hoffmann-LaRoche TAAR1-selective antagonist EPPTB with respect to blocking the locomotor activating effects of METH in wild type mice. We are currently exploring the effects of this compound on intravenous METH self-administration in wild type mice. Future studies will compare the locomotor and self-administration profiles of Taar1-deficient mice and their wild type littermates in the absence and presence of METH and our proprietary compounds.

Discussion: The goal of our research effort is to maximize TAAR1's therapeutic potential. To this end we are attempting to gain a more complete understanding of TAAR1 pharmacology and its signaling properties using an approach that is both integrative and multidisciplinary. Our results promote the further exploration of TAAR1 as a novel medication target for the prevention of relapse to METH abuse. Furthermore, as METH and its analogs are known to ameliorate the symptoms of attention deficit/hyperactivity disorder as well as promote wakefulness and enhance cognitive functioning, the appropriate pharmacologic manipulation of TAAR1 has the potential to benefit those suffering from a number of psychiatric conditions.

Disclosure: D. Grandy: none. **K. Tallman:** None. **M. Grandy:** None. **T. Wahl:** None.

211. Potent Reinforcing Effects of the Synthetic Cathinone Methylenedioxypyrovalerone (MDPV) in Rats

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Background: Synthetic cathinones are a class of designer drugs comprised of various amphetamine-like stimulants including 4-methylmethcathinone (mephedrone) and methylenedioxypyrovalerone (MDPV). Synthetic cathinones are frequently sold over the internet as "legal highs" or "bath salts", and produce psychostimulant effects by inhibiting presynaptic dopamine and norepinephrine transporters. Psychological effects of these drugs include euphoria, empathogenesis, and increased alertness and arousal. However, in recent years, poison control centers, emergency

rooms, and law enforcement agencies in the U.S. and elsewhere have witnessed a drastic increase in the number cases of adverse toxic effects of synthetic cathinones including tachycardia, hypertension, hyperthermia, psychosis, extreme agitation and violence, and death. The patterns of habitual use or abuse of synthetic cathinones and their potential for addiction have not been well-studied. We therefore sought to determine the reinforcing effects of MDPV using intravenous self-administration in rodents under short and extended access conditions. We also examined the effects of increasing behavioral demand on MPDV self-administration under a progressive ratio schedule of reinforcement.
Methods: Male Sprague-Dawley rats (250-275 g upon arrival) were implanted with jugular vein catheters and vascular access ports, and allowed to recover for at least 5 days. Following recovery from surgery, rats were placed in self-administration chambers and trained to press the active lever for delivery of 45 mg sucrose pellets on a FR1 schedule of reinforcement in a 16 hr overnight training session. Approximately 24 hr following the end of the initial training session, rats were then allowed to acquire intravenous self-administration of MPDV at doses of 0.05, 0.1 or 0.2 mg/kg per infusion (delivered in a volume of 0.06 ml over a 2 s period). These doses of MDPV were chosen based on our previous studies with i.v. self-administered methamphetamine (Gass *et al*., Neuropsychopharmacology, 2009). Each drug infusion was accompanied by illumination of a stimulus light above the active lever and presentation of an auditory stimulus ($\sim$65 dB, 2900 Hz) for 2 sec. Rats were first allowed 10 days of self-administration of MDPV under an FR1 schedule of reinforcement in daily 2 hr sessions. Rats then underwent testing under a progressive ratio (PR) schedule of reinforcement in a 16 hr overnight session. Next, for half of the animals, the length of the self-administration session was increased to 6 hr (long access, LgA), while for the other half of the animals the session length remained at 2 hr (short access, ShA). A total of 10 additional ShA or LgA access sessions were conducted.
Results: By the 4th self-administration session, all rats were able to discriminate between the active and inactive lever for MDPV reinforcement. Under both ShA and LgA conditions, rats demonstrated robust self-administration of MDPV. An inverse relationship between MDPV dose and the number of infusions obtained per session was observed on the 10th day of self-administration, with the mean number of infusions obtained being 35.8, 31.6, and 20.9 for the 0.05, 0.1, and 0.2 mg/kg doses, respectively. A liner relationship between MDPV dose and the number of infusions obtained under PR testing was observed, with the mean total number of infusions obtained being 4.5, 7.6, 14.4 for the 0.05, 0.1, and 0.2 mg/kg doses, respectively. Following 10 days of extended access to MDPV, rats self-administering the 0.05 mg/kg dose of MPDV did not show an escalation of intake, whereas rats self-administering the 0.1 mg/kg showed a 59% increase in total MPDV intake and rats self-administering the 0.2 mg/kg dose showed a 23% increase in total MPDV intake (p-values $<$0.05, day 10 vs. day 1 of LgA).
Discussion: Our findings indicate that MDPV is a potent reinforcer when self-administered intravenously, and produces rates of responding under ShA and PR conditions similar to those we have observed in rats self-administering methamphetamine (Gass *et al*., 2009). In addition, rats self-administering the higher doses MDPV demonstrated escalation of intake following extended access to the drug. Additional studies examining the effects of MDPV on brain stimulation reward are currently underway. Our findings suggest a potential for abuse of and/or addiction to MDPV, which carrries significant implications for the addiction community as well as future drug policy. This work was supported by NIH grant DA025606.
Disclosure: L. Watteron: None. **P. Kufahl:** None. **N. Nemirovsky:** None. **K. Sewalia:** None. **M. Olive:** None.

212. Behavioral and Thermoregulatory Effects of Novel Cathinone Derivative Drugs 4-MMC and MDPV
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Background: Methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (4-MMC) have emerged recently as popular recreational psychomotor stimulant compounds; they are often not distinguished from each other in popular reports as "bath salts". Very little information about substituted cathinones is available in the scientific literature but popular media reports have driven recent drug control actions in the UK and several US States.
Methods: Behavioral studies in rats used intravenous self-administration procedures and radiotelemetry monitoring of body temperature and activity. Additional studies determined the pharmacokinetics and metabolism of 4-MMC in rats as well as the effects of 4-MMC on dopamine (DA) and serotonin (5HT) accumulation in the nucleus accumbens.
Results: Both compounds were readily self-administered and induced locomotor stimulation, with MDPV exhibiting greater potency in dose-substitution procedures. Reductions in body temperature were produced by 4-MMC but MDPV did not affect thermoregulation. Rates of hepatic metabolism of 4-MMC were consistent with observed plasma clearance within about 90 minutes of injection. 4-MMC produced $\sim$9fold increase in DA and $\sim$22 fold increase in 5HT in the nucleus accumbens.
Discussion: These results show that the cathinone analogs 4-MMC and MDPV exhibit *in vivo* pharmacological properties that are distinct from each other. Reference to a larger literature on amphetamine stimulants suggests 4-MMC is more similar to 3,4-methylenedioxymethamphetamine and MDPV is more similar to methamphetamine.
Disclosure: S. Aarde: None. **M. Wright, Jr.:** None. **M. Buczynski:** None. **D. Angrish:** None. **L. Parsons:** None. **K. Houseknecht:** Part 1: Consultant for Biousian Biosystems, Inc. **T. Dickerson:** None. **M. Taffe:** None.

213. Opioid Regulation of GABAergic Circuitry in the Extended Amygdala
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Background: The kappa opioid receptor (KOR) and its endogenous agonist, the neuropeptide dynorphin, are a critical component of the central stress system. Both dynorphin and KOR are expressed in the bed nucleus of the stria terminalis (BNST) and the central nucleus of the amygdala (CeA), brain regions associated with anxiety and stress. Further, numerous studies suggest that the projection from the CeA to the BNST plays a crucial role in the regulation of anxiety and relapse-like behavior and contains dynorphin. These studies suggests that KOR activation in the BNST may play a role in the regulation of emotional behaviors such as relapse and anxiety. To date, however, there has been no investigation of the ability of KOR to modulate synaptic transmission in the BNST or specifically in this CeA to BNST pathway. Additionally, recent studies have highlighted the role that MAP kinases play in mediating the behavioral effects of KOR, however to date there is limited data on the role these signaling pathways play in KOR modulation of synaptic transmission.
Methods: We used whole-cell patch clamp recordings from acutely prepared mouse brain slices to examine the actions of KOR on inhibitory transmission in the BNST. Additionally, we used

neurochemical and pathway-specific optogenetic manipulations to selectively stimulate GABAergic fibers from the CeA to the BNST. In addition, we evaluted the role of ERK and p38 signaling on KOR mediated modulation of synaptic transmission in the BNST.
Results: We found that activation of KOR reduced GABAergic transmission through a presynaptic mechanism. Furthermore, we examined the signal transduction pathways that mediate this inhibition, and provide the first functional information implicating ERK in KOR-mediated presynaptic modulation. Moreover, we found that KOR signaling robustly reduced inhibitory synaptic transmission in the CeA to BNST pathway.
Discussion: Together, these results demonstrate that KOR provide important inhibitory control over presynaptic GABAergic signaling within the BNST, and provide the first direct functional demonstration of KOR sensitive long-range GABAergic connections between the CeA and the BNST.
Disclosure: K. Pleil: None. **C. Li:** None. **T. Kash:** None.

214. Stress Adaptations in Endocannabinoid Signaling in the Amygdala: Implications for Novel Treatment Approaches for Affective Disorders

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Background: Chronic stress is the primary environmental risk factor for the development and exacerbation of affective disorders, thus understanding the neuroadaptations that occur in response to stress is a critical step in the development of novel therapeutics for depressive and anxiety disorders. Brain endocannabinoid signaling is known to modulate emotional behavior and stress responses, and levels of the endocannabinoid 2-arachidonoylglycerol (2-AG) are elevated in response to chronic homotypic stress exposure. However, the role of 2-AG in the synaptic and behavioral adaptations to chronic stress is poorly understood.
Methods: We studied the effects of chronic restraint stress in male mice on synaptic and behavioral adaptations. We utilized whole-cell and field potential recordings to examine the synaptic adaptations in eCB-mediated signaling in the central and basolateral amygdala after chronic stress. Behavioral analyses were conducted after stress exposure and included novelty-induced feeding suppression, elevated plus-maze, and tail suspension test. To test the effects of augmenting 2-AG levels on stress-induced behavioral and synaptic adaptations, we treated mice with the monoacylglycerol lipase (MAGL) inhibitor JZL184 chronically during stress exposure. 2-AG measurements were performed by mass spectrometry.
Results: Our data show that in the basolateral amygdala, chronic stress exposure gates the induction of long-term depression of GABAergic transmission (LTDi). LTDi was presynaptically expressed and blocked by CB1 receptors, and reduced by inhibitors of 2-AG synthesis. This enhancement of LTDi was reversible after a week of recovery from stress exposure. Within the central amygdala, chronic restraint stress enhances both short- and long-term eCB-mediated synaptic depression at excitatory synapses. Depolarization induced suppression of excitation (DSE) was increased in magnitude after chronic stress, and long-term depression of excitatory transmission (LTD) was also enhanced after chronic stress. LTD in the CeA was reversed by application of a CB1 receptor antagonist, and absent in CB1 knock-out mice. These synaptic adaptations are likely mediated via downregulation of 2-AG degrading enzyme MAGL, and an upregulation of eCB synthetic capacity. Behaviorally, chronic restraint stress caused an anxiety-like phenotype that was prevented by chronic augmentation of brain 2-AG levels via administration of JZL184. This treatment prevented the stress-induced enhancement of eCB signaling at GABAergic synapses in the basolateral amygdala.
Discussion: These data suggest stress dynamically affects eCB synaptic plasticity in the amygdala, and that augmentation of brain 2-AG levels during stress can prevent some of the adverse behavioral consequences of stress exposure.
Disclosure: S. Patel: None.

215. Social Isolation during Adolescence Alters Dopamine Modulation in the Adult Medial Prefrontal Cortex.

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Background: The brain continues to develop throughout childhood and adolescence, and normal development can be disrupted by several external factors. Social interactions such as play behavior are crucial for the proper development of brain reward circuits, as well as social and cognitive functions. Baarendse *et al.* (Soc. Neurosci. Abs, 2011) have shown that social isolation in young rats leads to an impairment in inhibitory control under challenging conditions, and a blunted response to dopamine reuptake blockers when these rats have reached adulthood. Here, we tested whether these behavioral deficits were accompanied by changes in synaptic activity in the medial prefrontal cortex (PFC).
Methods: We examined whether three or five weeks of social isolation after weaning at postnatal day 21 lead to cellular or synaptic changes in the adult medial PFC using whole-cell recordings from pyramidal neurons in slices from adult rats.
Results: Social isolation rearing caused a loss of sensitivity to dopamine (DA) in these neurons. Electrical stimulation in superficial layers evokes excitatory post-synaptic potentials (EPSP) in layer V pyramidal neurons that were reduced in amplitude by a combination of D1 and D2 agonists in the majority of control rats (12 out of 15 cells). However, this reduction in EPSP amplitude was not readily observed in slices from rats that had been socially isolated either three or five weeks. Only 7 out of 32 cells showed a modulation by DA in isolated rats. These proportions were significantly different ($p = 0.0002$; Fisher Exact Test).
Discussion: A reduced attenuation of synaptic inputs to PFC pyramidal neurons by DA in adult rats that were socially isolated during juvenile and adolescent stages could yield altered PFC-dependent behaviors, including loss of inhibitory control and cognitive functioning.
Disclosure: D. Counotte : None. **P. Baarendse:** None. **L. Vanderschuren:** None. **P. O'Donnell:** None.

216. Essential Role of Ventral Tegmental Area Dopamine Neurons in Mediating the Induction and Rapid Reversal of Depression-Like Behaviors

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Background: The surprising rapid clinical effect of deep brain stimulation in depressed patients supports the notion that depression is a *neural circuit disorder*. In contrast, less is known if depression-like behaviors can be induced or reversed rapidly in animal models. Our previous *ex vivo* and *in vivo* studies showed that chronic social defeat consistently increased the firing rate and phasic firing events of ventral tegmental area (VTA) dopamine (DA) neurons in the brain reward circuitry of susceptible mice, but not of the resilient subgroup (*Cell* 2007; *J Neurosci* 2010). However,

the physiological function of this increased firing and its ionic mechanisms, as well as potential therapeutic targets, remain to be elucidated.

Methods: Susceptible and resilient mice were segregated following a chronic (10-day) social defeat paradigm, a well established model of depression. Utilizing optogenetic techniques – Cre-dependent expression of channelrhodopsin-2 (ChR2) in tyrosine hydroxylase (TH)-Cre mice, phasic firing was induced by high frequency optoactivation of ChR2 specifically in VTA DA neurons, and behavioral tests (social interaction and sucrose preference) were performed in behaving mice. The ionic mechanisms that underlie the hyperactivity of these neurons were also examined in TH-GFP mice by use of electrophysiological techniques.

Results: Consistent with our earlier findings, we found that optoactivation of ChR2 (mimicking phasic firing) during sub-threshold defeat, a paradigm that does not induce depressive-like behaviors in normal mice, increased social avoidance behavior and reduced sucrose intake. Importantly, the same optoactivation of these neurons during social interaction tests instantly induced similar susceptible behaviors in both resilient mice and the mice treated with sub-threshold defeat. These findings indicate that the phasic firing of VTA DA neurons encodes, and is tightly linked to, the susceptible phenotype. Next, toward understanding the ionic mechanisms of the higher VTA DA neuron firing, we previously found that the current of I_h, an important channel in the transition between tonic and phasic firing patterns, was upregulated in susceptible mice and that local infusion of I_h inhibitors ZD 7288 and DK-AH 269 into the VTA normalized depression-like social avoidance in susceptible mice within *one hour* after the infusion. This rapid effect is very different from classic antidepressants that take weeks to show treatment efficacy. Surprisingly, in our ongoing studies, we also observed that the antidepressant effect induced by a single-dose infusion of I_h inhibitor DK-AH 269 lasted at least two weeks. More importantly, this long-lasting effect was also seen with a single intraperitoneal dose of DK-AH 269. Following these studies, we also found that chronic defeat induced a larger increase in I_h current in resilient mice than in the susceptible subgroup. This finding suggests that the force driving the pathological hyperactivity persists in resilient mice, and that additional compensatory ionic mechanisms such as K^+ channels are necessary to drive the higher firing back to normal levels in resilient mice. Consistently, our data showed that K^+ currents were significantly increased selectively in resilient mice, which is consistent with our earlier microarray data. In addition, we found that local infusion of the K^+ (KCNQ) channel activator flupirtine into the VTA had similar rapid antidepressant effects as seen with I_h inhibitors. We are now investigating the effect of systemic administration of this activator and others.

Discussion: Here we demonstrate that optogenetics is a powerful tool to precisely imitate stress-induced physiological adaptations and reliably define VTA DA neurons in the brain reward circuit as a therapeutic target. Interestingly, optogenetically-induced phasic firing rapidly induces avoidance behavior during interaction tests, even in resilient mice. Our data also strongly support that I_h channels are one of the passive pathological ion mechanisms that underlie the susceptible firing increase, while K^+ channels are an important active ion mechanism that drives the pathological hyperactivity back to normal levels in resilient mice. Importantly, *passive* ion channel inhibitors and *active* ion channel activators that inhibit the higher pathological firing of VTA DA neurons are both antidepressant and pro-resilient. Our studies provide fundamentally novel drug targets for *rapid* or *long-lasting* antidepressants, which are mechanistically distinct from predominant monoamine-based medications.

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217. Elevation of Oxidative Stress in Tissue Cultures from Persons with Major Depression

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Background: Major depression (MDD) is a chronic and highly disabling condition with estimate to affect 15% of the population worldwide. The individuals with depression are at higher risk for chronic general medical conditions such as cardiovascular disease, obesity and diabetes. There is increasing evidence that chronic and severe medical conditions including major depression are associated with oxidative stress. Oxidative stress may be the result of diminished levels of antioxidants or increased production of reactive species from oxygen. Reactive oxygen species cause oxidative damage to lipids, nucleic acids, and proteins. We designed the experiments to determine if there is increased oxidative stress in tissue culture model of major depression.

Methods: Skin fibroblast samples were obtained by punch biopsy from persons with MDD and age-, race-, and sex-matched healthy controls (n = 14 each). All patients and controls provided written informed consent before taking part in the study. *Collection and processing of skin biopsy*. Specimens for fibroblast cultures were obtained by skin punch biopsies (1x2 mm). The tissue punch was cut into 4-6 smaller pieces and incubated in 0.5% trypsin-EDTA plus xy collagenase for 1 hr at 37°C. The full cell culture medium was added to the sample and spun at 90g for 5 min. Supernatant was discarded and 5 ml of fresh medium was added. The tissue was triturated and plated in 60 mm cell culture dishes. The medium was changed three times a week. In about 2-3 weeks, the plate was filled with fibroblasts. The cell cultures were expanded for freezing and used for experiments. *Cell cultures*. All cell lines were maintained in DMEM supplemented with L-glutamine, 10% fetal bovine serum (FBS; Thermo Scientific HyClone, Logan, UT), and penicillin/streptomycin at 37°C and 5% CO2. According to the vendor, the FBS contained cholesterol at concentrations of 32 mg/100 ml. This translates into a final cholesterol concentration of 32 μg/ml in our culture medium. For experiments in cholesterol-deficient serum, cells were cultured with medium containing 10% cholesterol-deficient serum (Thermo Scientific HyClone Lipid Reduced FBS). This FBS medium did not have detectable cholesterol levels. High-glucose media: high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose and 1mM sodium pyruvate and supplemented with 10% FBS, and penicillin-streptomycin. Galactose media: DMEM deprived of glucose supplemented with 10mM galactose, 2mM glutamine, 10% FBS, 1mM sodium pyruvate and pen-strep as above. *Protein oxidation determined by oxyblots*. Protein carbonyl levels were measured with the OxyBlot kit (Millipore) according to the manufacturer's recommendations. Carbonyl groups in the proteins were derivatized to 2,3-dinitrophenylhydrazine (DNPH). Western blot analysis was performed with an antibody to DNPH. Equal loading was assessed using an antibody against anti-GAPD (Cell Signaling). All blots were analyzed densitometrically.

Results: Cultured fibroblasts, control and MDD, are indistinguishable under both glucose and galactose cell culture conditions. They show similar gross morphological appearance when grown in either high-glucose medium or galactose medium. The proliferation rate was measured by qPCR using set of primers specific for Ki67, a proliferation marker. All cells showed similar expression level of Ki67 under both control and galactose conditions. In basal, high glucose medium, cultured fibroblast from MDD patients showed elevated levels of DNPH-labeled oxidized proteins compared to control. In galactose medium, cultured control fibroblasts had a marked up-regulation of oxidative stress compared to the glucose condition. Cells from MDD patients showed protein oxidation in the glucose condition that was

equivalent to galactose in the control samples; there was no up-regulation in galactose.
Discussion: Increased oxidative stress is present in the basal, glucose medium in fibroblasts derived from MDD patients relative to controls. Elevated oxidative stress may be the result of increased production of reactive oxygen species or reduced intracellular antioxidant status (e.g., glutathione). Although derived from peripheral tissues, the current findings may reflect altered redox regulation in persons with MDD.
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218. Deficits in vVesicle Priming Underlie decreases in Glutamate Release in Dysbindin-Mutant Mice

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Background: Schizophrenia is a relatively common neuropsychiatric disorder that involves debilitating and treatment-refractory cognitive deficits that significantly limit the psychosocial function of affected patients. The disorder is highly heritable, and a number of promising candidate susceptibility genes have emerged recently. Of the putative risk genes under study now, the gene encoding dystrobrevin-binding-protein-1 – *DTNBP1* – is of particular interest. *DTNBP1* lies within the chromosome 6p24-22 susceptibility locus and encodes the protein dysbindin. Dysbindin is expressed fairly ubiquitously throughout the brain where it is found in both pre- and postsynaptic neuronal elements. Amongst its functions, dysbindin is involved in the control of presynaptic release of glutamate and recent studies by us and others have reported that mutations in dysbindin elicit decreases in glutamate release in the PFC and hippocampus.
Methods: Whole-cell recordings in voltage clamp configuration were performed in 300 μm thick PFC slices of 45-60 days old male WT and dysbindin MUT mice.Electrodes (3-7 M resistance *in situ*) were filled with a solution containing (in mM): 135 CsCl, 10 HEPES, 2 $MgCl_2$, 1 EGTA, 4 NaCl, 2 Na-ATP, 0.3 tris-GTP, 1 QX-314, 10 phosphocreatine; 285 mOsmols. Series resistances (10-20 MU) and input resistances were continually monitored throughout the experiment . Pyramidal neurons were clamped at -80 mV. Electric stimulation was delivered via a bipolar concentric electrode positioned in layer 2 of the PFC. Evoked EPSCs were elicited via the stimulation electrode and the amplitude of the eEPSCs was adjusted to 75% of the maximum amplitude. A protocol consisting in 20 pulses at 40 Hz was delivered 30 times. Picrotoxin (50 μM) was included in the perfusion solution to block $GABA_A$ receptors. *Ca^{2+}currents*: All the voltage-clamp experiments were performed in the presence of 1 μM TTX and 20 μM TEA. Ten voltage steps from -100 mV to 60 mV (500 msec duration) were delivered 3 times and the average amplitude for every step was calculated Prefrontal cortex slices (250 μm) were labeled with FM1-43 (8 μM; Invitrogen) for 5 min in ACSF with 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) and then CNQX + FM1-43 in high $[K^+]_o$ (45 mM) and 2 mM Ca^{2+} for 15 min to stimulate uptake of FM1-43 via endocytosis of vesicles. After loading slices were washed out for 10 min prior to imaging. Slices were washed for 15 min in FM1-43 free ACSF containing 100íM sulforhodamin-101 and scanned. Depolarisation dependent destaining was obtained by application of 90 mM K^+. All FM1-43 experiments were performed using a Zeiss LSM 510 confocal laser-scanning microscope with a 40 × water dipping objective. The filtering strategy used an emission filter with a narrow band pass at 540 ± 20 nm, a range of wavelengths over which FM1-43 emits but S-Rhd does not.. To monitor, exocytosis, the brightness of single cell (containing clusters of synaptic vesicles) was quantified during all destaining or at specific time intervals. Additionally, synaptosomes were loaded with FM1-43 dye according to Meffert *et al* (1994). In brief, aliquots of WT, HET and dys MUT synaptosomes (0.4-0.5 mg of protein/ml) were resuspended in Krebs buffer and loaded with 5 μM of FM1-43 for 10 min at 30°C followed by the addition of 40 mM KCl for 1 min. Following loading, synaptosomes were pelleted by brief centrifugation followed by washing, repelleting, and resuspending in Krebs buffer containing 1mM CaCl2. Fluorescence measurements were carried out as described by Meffert *et al.* (1994).
Standard Western blots in a synaptosomal preparation were used to estimate level of proteins.
Results: Dysbindin MUT mice exhibit smaller amplitude eEPSCs, and a faster depletion of the ready releasable pool (RRP). When assessing Nq (number of vesicles), it was found that MUT mice have a 51% decrease in Nq (WT mice: 2445.9; MUT mice: 1250.9) when compared with WT mice. Moreover, in average, MUT mice also have a smaller probability of release (63% lower; WT mice: 2.63; MUT mice 1.66). Dys-MUT mice also exhibit a slower replenishment of the RRP. The time constant for recovery of the RRP in WT mice is 1.004 sec, and in dys-MUT mice is 1.179 sec. Dys-MUT mice exhibit smaller Ca^{2+} currents ($p < 0.02$) and a 43% reduction in FM1-43 uptake. Moreover, significantly reductions in the levels of syntaxin 1, synaptotagmin and synapsin were found. When Ca^{2+} influx was physiologically increased, glutamate release was improved.
Discussion: Our results suggest that Dys-MUT mice have a smaller RRP, and the RRP is depleted faster in dys-MUT mice than in WT mice, therefore resulting in the decreases in glutamate release reported in these mice. Moreover, dys-MUTmice have smaller Ca^{2+} currents, and together with decreases in the levels of synaptic proteins involved in the priming of synaptic vesicles, dysbdindin mutations produce deficits in neurotranmission . Preliminary results suggest that increasing Ca^{2+} influx it may restore glutamate release and it provides insights into the development of new therapeutics.
Disclosure: S. Saggu: None. **T. Cannon:** None. **J. Jentsch:** None. **A. Lavin:** None.

219. Acute Stress Alters Plasticity in the Nucleus Accumbens Via a Dopaminergic Mechanism

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Background: The neurophysiological response to stress involves the participation of multiple brain regions. The basolateral amygdala (BLA) and ventral subiculum of the hippocampus (vSub) convey emotion and context information, respectively, to the nucleus accumbens (NAc). The integration of inputs from the BLA and vSub in the NAc are likely central to coordinating behavioral responses to stressful experiences. In addition, alterations in the activation of the mesolimbic dopamine system have been observed following brief bouts of stress. Consequently, dopaminergic input to the NAc may be responsible for gating vSub and BLA input following stress.
Methods: Using in vivo extracellular recordings from NAc neurons exhibiting monosynaptic responses to stimulation of the BLA and vSub, we examined how acute restraint stress affects the plasticity of the vSub-NAc and BLA-NAc pathways. 24 hours prior to

electrophysiological recordings, animals in the acute stress group were placed in a Plexiglas restraint tube for 2 hours. Following baseline recording from NAc neurons, low-frequency stimulation (LFS, 500 pulses, 5 Hz) was applied to the vSub only. In a subset of cells, sulpiride, a D2-receptor antagonist, was applied iontophoretically either before or after low-frequency stimulation of the vSub. In separate groups of control and acute stress rats, the elevated plus maze was used to measure any stress-induced increases in anxiety behaviors. 24 hours after restraint stress, animals were placed on the elevated plus maze for 5 minutes and the number of entries to closed and open arms were recorded.

Results: In control animals, LFS of the vSub caused a prolonged decrease (>30 min) in responses to vSub stimulation without altering the responses to BLA stimulation. In addition, when sulpiride was applied 30 min after LFS, vSub-evoked responses remained suppressed. If sulpiride was administered prior to and during LFS induction, not only was the decrease in vSub-evoked responses blocked, there was instead a potentiation in vSub-evoked responses that was immediately eliminated when sulpiride was turned off. In addition, with sulpiride, LFS of the vSub caused a decrease in BLA-evoked responses in the NAc.

LFS to the vSub pathway after acute stress caused only a transient (<5 min decrease) in vSub-evoked responses. When sulpiride was administered 30 minutes after LFS in acute stress rats, there was a substantial increase in vSub-evoked responses. In contrast, when sulpiride was applied prior to LFS of the vSub in stressed rats, there was a pronounced decrease in vSub-evoked responses that persisted even after sulpiride was turned off. Interestingly, LFS to the vSub with sulpiride also caused a brief (<25 min) increase in BLA-evoked responses that was reversed after sulpiride was removed.

In comparison to control animals, restraint stress also caused an increase in the number of entries to the closed-arm portions of the elevated plus maze 24-hours after stress. This would suggest a stress-induced increase in anxiety behaviors simultaneous with the electrophysiological changes observed in the NAc.

Discussion: These data suggest that LFS of the vSub attenuates the efficacy of the vSub in driving the response of NAc neurons, and DA in the NAc shifts the vSub-BLA balance in favor of the BLA. Following acute stress, LFS is no longer effective in attenuating vSub-evoked responses. Indeed, following acute stress, DA now shifts the vSub-BLA input in favor of the vSub. As a consequence, the normal DA potentiation of affect-mediated gating is changed to a context-mediated response following stress. In altering the normal connectivity between BLA and vSub to the NAc, stress may therefore increase the expression of unwanted behaviors and lesson the likelihood of adaptive responses.

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220. Psychophysiological Predictors of PTSD Risk in Active Duty Marines: Preliminary Findings from the Marine Resiliency Study

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Background: Although physiological symptoms of arousal such as increased startle reactivity (DSM-IV) are among the most commonly reported symptoms by PTSD patients, surprisingly little is understood about the pathology underlying this symptom. This symptom is of interest for mechanistic studies because it is fairly selective to PTSD compared to other anxiety disorders. Two recent studies suggest that startle potentiation when anticipating mildly aversive stimuli is associated with subsequent risk for development of PTSD in civilian populations, suggesting that increased startle reactivity during mild or ambiguous threat may be a risk factor for PTSD. Here we tested the hypothesis that increased arousal as measured by startle reactivity may predict risk for combat-induced PTSD using a longitudinal design.

Methods: To test our hypothesis, we examined startle responses in active duty Marines before deployment and 3 months after deployment to Iraq or Afghanistan. This study was conducted as part of a 4 hour test battery that encompassed clinical assessments for PTSD, psychosocial scales, laboratory measures and psychophysiological measures that is conducted both before deployment as well as 3 months and 6 months after deployment (Marine Resiliency Study) in >2000 active duty marines. Physiological responses examined were startle magnitude, threshold and habituation, as well as affective modulation of startle in response to combat-related imagery. Here we will present some initial analyses of the current data set. We examined (1) effect of deployment overall on physiological reactivity measures (comparison of pre-deployment to 3-months post deployment time points, N = 1345) on baseline startle, prepulse inhibition and affective modulation of startle), and (2), comparison of pre-deployment startle reactivity across subjects matched for combat exposure with and without PTSD symptoms at the 3-month time point (n = 47-49/group, PTSD group defined by PTSD Checklist Score > 44 at 3 months post deployment).

Results: When analyzing across all subjects, we observed small but significant increases in both baseline startle reactivity and prepulse inhibition after deployment (Main effect of deployment, $p < 0.001$). Startle reactivity while viewing combat images was also significantly increased after deployment (Main effect of deployment $p < 0.001$). No effects of deployment on startle habituation were observed. Baseline startle magnitude *before* deployment was significantly greater in subjects that went on to develop PTSD after deployment compared to their combat-matched controls ($p < 0.05$ post hoc planned comparison following significant interaction between deployment × PTSD × startle block). To control for changes in hearing all analysis were conducted using hearing test performance as a covariate.

Discussion: These preliminary data suggest that deployment experience has subtle effects on physiological reactivity both at baseline and in response to combat-related cues. Analysis is ongoing to determine if deployment effects are related to combat experience and/or psychosocial changes after deployment in some individuals. In addition, the preliminary results from combat PTSD and matched controls support previous reports suggesting that startle reactivity may probe trait biological processes that confer risk for PTSD.

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221. Unique Changes in Fast-Spiking Interneuron Activity during Sleep-Dependent Consolidation of Plasticity in the Developing Visual Cortex

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Background: Ocular dominance plasticity in the visual cortex (V1) is a canonical form of *in vivo* synaptic plasticity, which is initiated during a critical period of postnatal development by waking monocular visual experience, and consolidated by subsequent sleep. Our previous work has shown that the visual cortex undergoes changes in network activity during the first hours of post-monocular deprivation sleep. These changes are associated

with functional potentiation of non-deprived eye responses in V1 neurons, and cellular changes similar to those involved in long-term potentiation (LTP) of glutamatergic synapses.
Methods: Using chronic single-unit recording of individual V1 neurons in freely-moving, freely-sleeping cats, we were able to characterize how waking visual experience and subsequent sleep affect activity patterns in fast-spiking (FS; presumptive GABAergic) and non-FS (presumptive pyramidal) neurons. Mean firing rates and burst rates were quantified for each recorded neuron across 6 hours of waking experience (either control binocular visual experience, monocular deprivation, or binocular recovery from extended monocular deprivation - which initiates a form of plasticity not dependent on sleep), and subsequent rapid eye movement (REM) and non-REM sleep.
Results: We observed that monocular deprivation leads to a gradual decrease in activity among FS interneurons, which is maintained during the first few hours of subsequent sleep. Similar changes in FS neuron activity do not accompany normal binocular visual experience, or during ocular dominance changes associated with binocular recovery from monocular deprivation (which do not require sleep). Importantly, non-FS neurons become more active during sleep-dependent consolidation of ocular dominance plasticity following monocular deprivation (but not following other forms of visual experience). Increases in the firing rate and burst rate of non-FS neurons during post-monocular deprivation sleep are proportional to their changes in ocular dominance, and potentiation of non-deprived eye responses, during the same periond.
Discussion: We find that during sleep-dependent consolidation of ocular dominance plasticity, which is marked by LTP-like changes in V1, there is a marked alteration in the balance of activity among putative excitatory neurons and interneurons. The activity of interneurons becomes suppressed during monocular visual experience, and continues to be suppressed over the first few hours of subsequent sleep. At the same time, activity in pyramidal neurons is enhanced, and this enhanced activity correlates with response potentiation during sleep. Thus one possibility is that depression of interneuron activity results in disinhibition of pyramidal neurons during sleep, which drives sleep-dependent plasticity in V1 circuits.
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222. The Roles of the Infralimbic Cortex in Impulsive Action

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Background: Higher impulsivity could be a risk factor for drug addiction, criminal involvement, and suicide.Moreover, poor inhibitory controlis often observed in several psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD), schizophrenia, substance abuse, bipolar disorder, and borderline personality disorder. Thus, it is of interest todevelop treatments for these disorders to suppress impulsive acts, and toelucidatethe neural basis of the inhibitory control of impulsive action. We recently found that milnacipran, an antidepressant and a serotonin/noradrenalin reuptake inhibitor, could augment impulse control in rats. Milnacipran increases not only extracellular serotonin and noradrenalin but also dopamine specifically in the medial prefrontal cortex (mPFC) where one of brain regions responsible for impulsive action. Therefore we examined whether dopamine D1-like and/or D2-like receptors in the infralimbic cortex (IL), the ventral portion of the mPFC, mediate the milnacipran-enhanced impulse control in the three-choice serial reaction time task (3-CSRTT). To further elucidate the neurophysiological basis of impulse control, we recorded single unit activity in the IL of a rat performing the 3-CSRTT.
Methods: Male adult Wistar/ST rats were used.After completing the training of 3-CSRTT, rats were divided into the two groups: A group of rats were bilaterally injected with SCH23390, a selective D1-like receptors antagonist, (0.3 or 3 ng/side) or eticlopride, a selective D2-like receptors antagonist, (0.3 or 1 í g/side) into the IL after acute injection of milnacipran (10 mg/kg, i.p.). The test session of 3-CSRTT was started 30 min after the injection of milnacipran. For another group of rats, a microelectrode consisting of four tungsten wires (25 ím diameter, Calif̀ornia Fine Wire) with gold-plated tips (DC resistance, 200-600 kÙ) was implanted into the IL. The microelectrodes were equipped with a custom-made microdrive, which allowed the detection of neural activity by extending the electrode tips after surgery.
Results: We found that intra-IL SCH23390 infusions reversed the milnacipran-improved impulse control, while infusions of eticlopride into the IL failed to block the effects of milnacipran on impulsive action. Thus, D1-like but not D2-like receptors in the IL have a critical role in milnacipran-augmented control of impulsive action. Of the 56 neuronal units that were isolated from 8 rats in the present study, 25 units were linked to impulse control, while 4 units were linked to attentional performance. In addition, 13 units were linked to both impulse control and attentional performance. We found that above half of the recorded neurons (38/56) were linked to impulse control.
Discussion: Taken together with these results, we concluded that the rat IL plays an important role in modulating the inhibitory control of impulsive action. To explore/develop the treatments modulating neural activities in the IL could provide therapeutics to suppress impulsive behavior.
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223. Safety Learning requires Synaptic Plasticity in the Posterior Insular Cortex

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Background: Veridical detection of safe versus dangerous environments is critical to survival. We reported that the posterior insular cortex (IC) is critical the stress-mitigating effect of safety signals on later anxiety behavior. The current study examined plasticity in this region as rats learn a safety signal.
Methods: Rats received "A + /B-" fear discrimination training in a standard fear-conditioning box. Training consisted of 15 A + trials where a distinct auditory cue preceded a brief footshock. Intermixed with A + trials were 15 B- trials that were not reinforced. Trials began with a 5 sec house light on a 90 sec inter-trial-interval. 24 h after training rats were given a 3 minute summation test in the training context in which freezing, an index of fear, was assessed during one minute of each: the context alone, context and A, and context with A and B in compound. In Experiment 1 the NMDA-receptor antagonist AP-5 (6ug/side) was injected to the IC prior to training. In Experiment 2 rats were trained and sacrificed for tissue collection after 1 or 4 days of training. Phosphorylated extracellular regulated signaling kinase (pERK) was quantified using western blot from IC and other regions of interest.
Results: After three days of discrimination training presentation of the B cue significantly reduced freezing in the presence of A in the

summation test. In other words, A became a danger signal and B became a safety signal. The magnitude of this safety signal effect increased with up to 5 days of training at which point B reduced freezing to baseline levels. In Experiment 1, intra-IC AP5 injections significantly impaired discrimination learning such that after 5 days of training B did not significantly reduce freezing to A. Rats that received AP5 received additional drug free training and appeared to learn the A/B discrimination. Examination of IC pERK is ongoing; preliminary results suggest that the ratio of pERK2 to total ERK2 increases with training.
Discussion: These data suggest that synaptic plasticity in the posterior IC is critical for safety learning. Traditionally the IC has been thought of as a point of sensory integration; anatomically, the IC is positioned to provide rich sensory information to the amygdala, which is the proximal mediator of fear learning and expression. A growing literature suggests that safety learning is impaired in PTSD populations and functional and structural abnormalities have been reported in the IC of persons with PTSD and other mood disorders. Thus these data provide a link between these clinical findings and offer a novel target for preventive and therapeutic development. Ongoing studies investigate the impact of stress on safety learning and recall and plasticity within the IC and amygdala circuit.
Disclosure: J. Christianson: None. **J. Flyer:** None. **L. Watkins:** None. **S. Maier:** None.

224. The Impact of Multi-System Infections on the Regulation of Affective States is Explored using Chronic Neurologic Lyme Disease as a Model

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Background: Multi-system infections are hypothesized to be important pathogenic factors in diseases such as systemic lupus erythematosus, rheumatoid arthritis and chronic Lyme disease in which depression and anxiety are comorbid conditions that are difficult to explain. In the latter disease, referred to as post-treatment Lyme disease syndrome (PTLDS), depression and anxiety are cardinal symptoms. In clinical practice most patients with acute Lyme disease respond well to antibiotic treatment. On the other hand, Lyme patients whose disease isn't detected until later, when the Borrelia burgdorferi (Bb) spirochete has disseminated to other places, such as the central nervous system, are at greater risk of having residual neurological and neuropsychiatric symptoms. We performed a retrospective analysis of patient data collected in our Ceftriaxone retreatment study (Fallon 2007) to determine whether neuropsychiatric symptoms are associated with compartment-specific antibody production (CSF vs serum) and/or abnormally activated brain regions as measured by [^{18}F]-Fluorodeoxyglucose (FDG) brain PET.
Methods: We modeled baseline neuropsychiatric symptoms using serum Bb antibody level and regional brain neurofunction, including the interaction of these biological factors with intrathecal antibody (Ab) status. Serum and CSF samples and FDG PET images were available for 25 of 37 patients from our retreatment study of PTLD patients. All patients met the CDC criteria for Lyme diagnosis and had positive IgG Western blots. We measured antibody reactivity against the C6 peptide of the VlsE protein of B. burgdorferi in serum and CSF by ELISA (Immunetics Inc). Intrathecal Ab production (C6 AI) was estimated using the formula: CSF/serum ratio of C6 Ab divided by the CSF/serum ratio for total IgG. Fifteen patients were positive (+ : C6 AI $>$ 1.5) and 10, negative (–: C6 AI 1.0). In an initial data reduction step, patient FDG PET images were reduced to three fixed spatial patterns of covarying regional metabolism, representing the major differences in neurometabolism among patients (Moeller and Habeck, 2006). Alterations in the association between serum C6 Ab level, neurometabolism and neuropsychiatric symptoms were examined, and the differences associated with C6 AI status were quantified. The aim was to account for symptom severity on a patient-by-patient basis; standard statistical methods were applied to assess accuracy.
Results: At baseline the C6 AI + and C6 AI– patient groups showed significant neuropsychiatric symptoms and cognitive deficits, but did not differ in these domains. A single model fit patient scores on two standardized neuropsychiatric scales (NPS, $p < .005$): Beck Depression Inventory and the Zung Self-rating Anxiety Scale. The model scores were also significantly correlated with the Fatigue Severity Scale and McGill Pain Questionnaire ($p < .05$). The model involves a coupling between metabolic rate in particular brain regions and serum Ab level, and includes an interaction of these factors with intrathecal Ab status. Brain regions include hypothalamus, thalamus, anterior cingulate and bilateral amygdala and insula. Without the brain PET data the correlation between NPS and fluid immunoassays is not clinically significant. Moreover the composite serologic/imaging biomarker is specific to NPS and is not correlated with cognitive symptoms.
Discussion: We combined a blood serum immunoassay with functional brain imaging to produce the first tangible linkage to neuropsychiatric symptoms and their severity in chronic neurologic Lyme disease. A number of brain regions were affected. These areas are potential sites of past or current local infection or immune activation, or correspond to one or another brain circuit whose neurofunction has been altered -at multiple, spatial distributed circuit nodes. Our preliminary findings suggest the involvement of whole neural pathways regulating innate immunity (hypothalamic circuitry) and pathways associated with pain and mood regulation (amygdala and insula circuitry). In addition the neurofunction affected is different depending upon the level of intrathecal Ab production. In acute Lyme disease elevated intrathecal Ab production is an indicator of borrelia dissemination to the central nervous system. We now have evidence that in C6 AI + PTLD patients, the elevation of intrathecal Ab production is likewise associated with alterations in brain regions and circuits. In sum, composite serologic/brain image modeling offers a new approach to exploring the impact of multi-system infections on the regulation of affective states.
Disclosure: J. Moeller: None. **B. Fallon:** None. **J. Keilp:** None.

225. TRP Channels as Mediators of Oxytocin-Induced Anxiolysis

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Background: Transient receptor potential (TRP) channels are non-selective cation channels that are activated by a variety of mainly sensory stimuli. The TRPV channel subfamily members 1-4 were originally described as thermosensors in tissues concerned with thermoregulatory homeostasis, but they are also expressed in tissues that should not experience any temperature swings, such as the brain. Here, they serve probably other functions, which have remained elusive until now. TRPV2 channels are substantially and specifically expressed in the hypothalamic paraventricular, supraoptic, and suprachiasmatic nuclei, especially in vasopressinergic and oxytocinergic circuitries. This restricted distribution of TRPV2 channels makes it possible to address their physiological roles in oxytocin- or vasopressin-mediated behavior and physiology quite precisely. Here, we studied the role of TRPV2 channels on oxytocin-mediated

anxiolysis that we have previously localized to the paraventricular nucleus (PVN).
Methods: First, we set up a rat primary hypothalamic cell culture, and found, by calcium-imaging, that some cells showed Ca^{2+}-oscillations, whereas others did not. Second, we assessed whether oxytocin (100 nM) modulates intracellular Ca^{2+} (Ca_i^{2+}) signaling in these cells, and if so, whether Ca^{2+} from intra- or extracellular sources is involved. Potential Ca^{2+} influx through TRP channels was assessed by including the TRPC6, TRPC7, TRPV2 blocker SKF96365 (100 μM) in the cell culture medium. Third, we assessed the role of TRPV2 activation on oxytocin-induced anxiolysis, in rats that received SKF96365 bilaterally in the PVN at 0.5 nmol / 0.5 μl. Anxiety-like behavior was measured on the elevated plus maze (EPM) and in the light/dark box (LDB).
Results: Oxytocin appeared to influence only the oscillating cells, and increased intracellular Ca^{2+} (Ca_i^{2+}) concentrations, along with increased amplitudes of the Ca_i^{2+} waves (t-test, $p<0.05$). Thapsigargin (1 μM) failed to influence both Ca_i^{2+} concentrations and oscillation patterns, thus excluding the involvement of intracellularly-stored Ca^{2+} in the responses to oxytocin. In the absence of extracellular Ca^{2+}, Ca_i^{2+} oscillations disappeared, the Ca_i^{2+} concentration dropped significantly, and oxytocin was without effect on Ca_i^{2+} oscillations and concentrations, indicating that oxytocin facilitates the influx of extracellular Ca^{2+}. To test whether oxytocin-stimulated Ca^{2+}-influx is mediated by TRPV2 channels, cells were incubated with SKF96365. SKF96365 alone did not affect any of the parameters measured, but in the presence of oxytocin, it fully abolished the Ca_i^{2+} oscillations and reduced Ca_i^{2+} concentrations to 35% of pre-drug levels (t-test, $p<0.05$). It thus not only appears that oxytocin mediates its effects through TRPV2 or perhaps TRPC6 channels (PCR analysis demonstrated that TRPC7 is not expressed in our primary cell culture), but also that it stimulates the efflux of Ca_i^{2+} to the extracellular space in parallel. This probably serves to compensate for the Ca_i^{2+} influx normally seen in the presence of oxytocin, and to maintain Ca_i^{2+} homeostasis. Importantly, local infusion of SKF (0.5 nmol / 0.5 μl) in the PVN prior to oxytocin infusion (0.01 nmol 0.5 μL) prevented the anxiolytic effect of oxytocin as measured on the EPM and in the LDB (ANOVA, $p<0.05$), indicating that oxytocin reduces anxiety-like behavior in rats via Ca^{2+}-influx through TRPV2 (or TRPC6) channels.
Discussion: The present findings implicate, for the first time, a TRPV channel in the control of mammalian behavior, and show that its activity is controlled by a neuropeptide. This novel mechanism explains how the anxiolytic activity of oxytocin in the PVN is brought about, and might be of interest in the quest for efficient therapeutic strategies to treat anxiety or other affective disorders.
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226. Reduced Play Behavior and Altered Monamine Neurochemistry in Fisher 344 Rats

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Background: Play in some form occurs in the young of most mammalian species. The exact function of play still remains elusive, yet there is general consensus that removing the opportunity to play can have a number of consequences on later behavior and social functioning. Adolescent Fischer 344 rats are known to be less playful than other rat strains, although the neurobiological substrate(s) responsible for this phenotype is uncertain. In the present study, Fischer 344 rats were compared to the commonly used outbred Sprague-Dawley strain on behavioral and neurochemical measures in order to ascertain whether the lack of play may be related to compromised activity of brain monoamine systems.
Methods: Male Sprague-Dawley ($n=28$) and Fisher 344 ($n=28$) rats were assessed for play behavior between 30 and 40 days of age in two five min tests. Play bouts were recorded as digital video files and scored later using behavioral observation software. Play was quantified by counting the frequency of contacts directed by the test rat towards the nape of the target Sprague-Dawley rat (nape contacts) and the likelihood that a nape contact directed by the target Sprague-Dawley rat to the test rat resulted in a response. One week after testing for play behavior, rats were tested for acoustic startle response and pre-pulse inhibition of that response. A single session of 54 trials was used to assess baseline startle, habituation, and pre-pulse inhibition. A second set of Sprague-Dawley ($n=7$) and Fisher 344 ($n=7$) were used to measure levels of monoamine and metabolite levels in the striatum and prefrontal cortex using high performance liquid chromatography with electrochemical detection (HPLC-EC).
Results: As expected, Fischer 344 rats were far less playful than Sprague-Dawley rats, with Fischer 344 rats less likely to initiate playful contacts with a playful partner and less likely to respond playfully to these contacts. We also found that Fischer 344 rats showed less of a startle response and greater pre-pulse inhibition (PPI), especially at higher pre-pulse intensities. Monoamine analyses revealed Fischer 344 rats to have less dopamine and serotonin turnover in the striatum and prefrontal cortex but higher norepinephrine turnover.
Discussion: These data indicate that deficits in play and enhanced PPI of Fischer 344 rats may be due to dysfunctional modulation of corticostriatal and mesocortical circuits critical to the execution of these behaviors. These data also suggest that Fisher 344 rats may be useful for better understanding aspects of disorders where normal playful interactions are impaired (e.g. autism and ADHD) and in identifying the neurobiological substrate(s) associated with that specific impairment.
Disclosure: C. Crawford: None.

227. Dopamine Axons Innervating the Rat Lateral Habenula: Ultrastructural Features Common to and Distinct from Other Forebrain Dopamine Projections

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Background: The lateral habenula (LHb) serves negative reinforcement learning, providing a mechanism for suppressing responses to negative stimuli and facilitating behavioral switching. Structural and functional changes in the LHb have been identified in major depressive disorder and in animal models of depression. The LHb maintains prominent projections to brainstem monoamine neurons and potently suppresses their firing through a disynaptic mechanism. Interestingly, dopamine (DA) neurons in the ventral tegmental area send a reciprocal projection back to the LHb and modulate neuronal activity in this structure. We sought to investigate the ultrastructural features of this mesohabenular pathway and the potential anatomical substrates for DA modulation of LHb afferent control.
Methods: Sections through the LHb of four rats were labeled by immunoperoxidase for the DA synthetic enzyme tyrosine hydroxylase (TH). The medial parvocellular subnucleus of the LHb contains the densest DA innervation and was therefore examined by electron microscopy.
Results: Approximately 16% of the TH-immunoreactive (-ir) profiles were myelinated axons, a higher proportion than observed in any other forebrain region. Unmyelinated fibers of passage were also observed, and approximately 8% of TH-ir varicosities contained one or more dense-cored vesicles. TH-ir profiles frequently formed symmetric synaptic contacts onto the dendrites of LHb cells; only rare asymmetric synapses were seen. Many of the

target dendrites received additional synaptic input from unlabeled axons. TH-ir varicosities ranged in short axis diameter from 0.26 to 1.49 ím (0.63 ± 0.23; mean ± stdev, N = 298), and their target dendrites had cross-sectional diameters ranging from 0.26 to 3.14 ím (0.96 ± 0.52, N = 166).

Discussion: These findings indicate that the mesohabenular DA input is likely to: 1) exhibit a faster conduction velocity than nigrostriatal, mesolimbic, or mesocortical pathways, 2) mediate an inhibitory influence on LHb cell firing at both distal and proximal dendrites, and 3) modulate the afferent regulation of LHb neuronal activity. The results have important implications for understanding the reciprocal control between the LHb and midbrain DA neurons and the contribution of altered activity in this circuitry for mental disorders.

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