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Poster Session II

1. Spatial Distribution of Glycolysis as Measured by PET Images of Glucose and Oxygen Metabolism in the Resting Healthy Human Brain Correlates with Distribution of Beta-Amyloid Plaques in Alzheimers Disease

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Background: The distribution of beta-amyloid (Aβ) plaques in individuals with dementia of the Alzheimer type (DAT) can be visualized using PET and the tracer [11C]PIB (Klunk et al, Ann Neurol, 2004). However, the distribution of Aβ plaques is not uniform and may relate to underlying brain metabolism or function (Buckner et al, J Neurosci, 2005). We and others have shown that physiologic neural activation in the human brain is associated with greater increase in local consumption of glucose than oxygen, indicating a shift to glycolysis (Fox et al., Science, 1988). We now argue that using PET measures of cerebral metabolic rate of oxygen (CMRO2) and glucose (CMRGlu) obtained in the resting human brain reveals a spatial distribution of glycolysis that reflects baseline levels of neuronal activity. This study compares the spatial distribution of Aβ plaques seen in DAT subjects with the distribution of glycolysis in resting healthy subjects.
Methods: We examined the spatial correlation between Aβ plaques in 11 individuals with DAT (mean age = 79 ± 5 yrs) and glycolysis in a group of 16 healthy controls (mean age = 50 ± 14 yrs). Images representing the distribution of Aβ plaques were created from PET scans obtained from 30 to 60 minutes after injection of [11C]PIB. PET images were summed, normalized by cerebellar activity and then averaged in Talairach atlas space after normalization. Images representing the level of glycolysis at baseline were generated from sequential PET scans of three [15O] tracers (CO, O2 and H2O) and [18F]FDG. CMRO2 and CMRGlu were estimated from the [15O] tracers and the [18F]FDG, respectively, using a nonquantitative approach. Consumption of glucose in excess of that expected by the level of oxygen consumption is a marker of glycolysis. Thus, individual images of glycolysis were calculated from the pixel-by-pixel positive residual after regressing the estimates of the glucose to the oxygen consumption. Similarly to the [11C]PIB data, the glycolysis images were then averaged in Talairach atlas space. Two different methods were then used to sample the Aβ plaque image and the glycolysis image for correlation analyses. First, the entire brain was exhaustively sampled using adjacent non-overlapping 12 mm cubes. Second, a representative series of cortical spherical regions-of-interest were placed throughout both image sets.
Results: The mean images of Aβ plaques and glycolysis were highly correlated as evaluated by either complete brain sampling by non-overlapping 12 mm3 cubes ($r = 0.669$, $p < 0.0001$, n=729) or a set of cortical regions-of-interest spheres ($r = 0.804$, $p < 0.001$, n=50).
Discussion: Visually, glycolysis was low (i.e., there was little excess CMRGlu compared to CMRO2) in areas with low PIB binding (e.g., cerebellum, insular cortex) while glycolysis was high in areas of high PIB binding (e.g., precuneus, prefrontal cortex). This preliminary topographical relationship between resting glycolysis in controls and Aβ plaques in DAT individuals implies a link between resting brain metabolism and the later development of Alzheimer's pathology. This research is support by grants from the NIH (NS006833, NS048056, AG026276, AG05681, AG03991).

2. Assessing Health Related Resource Use and Value of Time Among Healthy Elderly: Baseline Data from the ADCS Prevention Instrument Project

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Background: Resource utilization and costs for healthy, cognitively intact elderly as they begin to demonstrate cognitive deterioration are not well understood. Also, value of time for participation in work (volunteer or paid) is often disregarded in elderly populations. The purpose of this study was to evaluate the utility of the Resource Use Inventory (RUI) developed by the Alzheimer's Disease Cooperative Study (ADCS) and assess resource and time use in a sample of non-demented elderly individuals living in the community.
Methods: The RUI was administered either at home or in the clinic to 644 healthy, non-demented individuals age≥75 and their study partners. The RUI quantified subjects' use of direct medical care (e.g., hospitalizations), non-medical care (e.g., home health aides), time spent by paid and unpaid caregivers providing care to the subjects, and subjects' participation in volunteer work and paid employment. Assessment interval for each question was the past three months. Quantified measures of usage for each domain of care were converted into monetary values based on average prices reported from public sources. National average hourly earning for all private industries was used to estimate hourly wage rates of unpaid caregiving costs and time spent in volunteer or paid work. Subjects' demographic characteristics included age, race/ethnicity, gender, and education. Clinical characteristics included baseline Clinical Dementia Rating (CDR), modified Mini-Mental State Examination (mMMS), activities of daily living (ADL), and whether subjects demonstrated cognitive deterioration over 24 months. Resource use and costs were compared by demographic and clinical characteristics, and partner participation. Comparisons of utilization and costs were performed using t-tests and Wilcoxon ranksum tests.
Results: Utilization of resources varied widely by type: 76.6% were examined at least once by a doctor, 10% received non-paid care, and 2% received paid care. Half of the subjects (51.6%) were engaged in volunteer work and 14% in paid employment. Among the users of health care resources, the most costly resource items included hospitalizations ($15,970) and imputed cost of non-paid care ($2,688). The value of time spent by the subjects in paid and volunteer work were estimated at $3,204 and $1,135. For the sample as a whole, three-month total cost was estimated at $2,288 per person. The most costly resource item was hospitalizations ($571). The value of time spent by the subjects in paid and volunteer work were estimated at $423 and $634. Total cost was significantly higher for men than women ($2,843 vs. $1,890) and for subjects with higher education level than those with lower education ($2,697 vs $1,864). Hospitalization costs were not significantly different by demographic and clinical characteristics measured. The dollar value of paid work was significantly higher for men ($760 vs $215) and the value for volunteer work was significantly higher for those with higher education ($725 vs $538), better CDR ($698 vs $417), better ADLs ($690 vs $439), and for subjects who did not demonstrate cognitive deterioration ($652 vs $300).
Discussion: Self-reported resource use in non-demented elders is feasible for tracking resource and time use through transition from healthy aging to cognitive impairment. Results illustrate that value of time for paid and volunteer activities are sensitive to cognitive changes even in elderly populations.

3. MEM 1003, a Novel, CNS-Selective L-Type Ca2+ Channel Blocker, Is a Potential Therapeutic Agent for CNS Disorders

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Sponsor: Eric R. Kandel

Background: Neurons must maintain low intracellular concentrations of Ca2+ to function properly. Neurons lose this ability as they age or as they are affected by disease. An increase in intracellular Ca2+ alters the activity of Ca2+-dependent signaling pathways which may adversely affect neuronal functions. One such Ca2+-mediated signal that may contribute to the decrease in cognitive performance associated with aging is an enlarged slow afterhypolarization (sAHP) due to the activation of Ca2+-dependent K+ conductance. Our recent findings demonstrated that sAHP amplitude co-varies with spatial learning ability in aged rats. Excessive intracellular Ca2+ may be exacerbated by an increase in density of L-type Ca2+-channels that reportedly occurs with aging and in Alzheimer's disease. Blocking voltage-regulated calcium channels facilitates the ability of neurons to maintain appropriate calcium levels and to function properly. Memory Pharmaceuticals is developing MEM 1003, a dihydropyridine (DHP) L-type Ca2+ channel blocker, for Alzheimer's disease and bipolar disorder.
Methods: Object Location Memory: Rats were allowed 15-minutes of exploration of three identical training objects. After a 6-hour delay, rats were tested for spatial memory. For the test, three new objects were placed in the chamber: two in the location used during training, one moved to a novel location. The rats were allowed to actively explore until they accumulated a total of 60 seconds of object exploration.
Results: MEM 1003 is a novel member of the L-type Ca2+ channel blocker class of drugs because of its preferential effects on CNS tissue versus its effects on cardiovascular tissues. MEM 1003 is effective in improving cognitive performance in a variety of preclinical behavior models including object location memory with aged rats and a transgenic mouse animal model of Alzheimer's disease. The effects of MEM 1003 across these models occur at plasma exposure levels that are consistent with the affinity of MEM 1003 for the DHP binding site. In contrast to other L-type Ca2+ channel blockers, MEM 1003 does not relax smooth muscle or reduce blood pressure at exposure levels necessary for cognitive improvement. Moreover, MEM 1003 lacks potency in reducing tension in pre-contracted human coronary artery tissue in an ex vivo assay. In addition, MEM 1003 does not reduce blood pressure at doses used in the clinical Phase 1a and 1b trials. A possible mechanistic explanation for the CNS-selective properties of MEM 1003 is that this compound is more potent in blocking Ca2+ conductance at depolarized potentials than at resting membrane potentials. This means that MEM 1003 may be more effective in blocking channels expressed in excitable cells that depolarize frequently, such as neurons, than in non-excitable cells such as smooth muscle cells. We will present data demonstrating the voltage-dependent inhibition properties for MEM 1003.
Discussion: The pharmacokinetic and safety profiles of MEM 1003 were recently studied in double-blind, randomized, placebo-controlled Phase 1A (healthy volunteers) and Phase 1B (patients with Alzheimer's disease) clinical trials. MEM1003 was well tolerated up to the highest dose tested of 180 mg twice daily. The safety data and pharmacokinetics of MEM1003 in these studies will be presented and the relevance of the exposure levels to preclinical animal efficacy models will be shown. MEM 1003 is currently in a Phase 2A clinical trial for Alzheimer's disease and for bipolar disorder. There is a large unmet need to address the treatment of CNS disorders that involve perturbations of Ca2+ signaling; because of the unique properties of MEM 1003, this drug is an excellent candidate to address that need.

4. Salvinorin A: Allosteric Interactions at the mu Opioid Receptor

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Background: Salvinorin A, a neoclerodane diterpene, is a kappa opioid receptor agonist that lacks the usual basic nitrogen atom present in all other known opioid ligands. Our initial binding experiments indicated that Salvinorin A weakly inhibited mu receptor binding. Additional experiments showed that Salvinorin A partially inhibited mu receptor binding. We therefore hypothesized that Salvinorin A allosterically modulates mu receptor binding.
Methods: To test this hypothesis, we conducted opioid binding assays and [35S]-GTP-γ-S binding assays using membranes prepared from CHO cells expressing the cloned human opioid mu receptors.
Results: Salvinorin A partially inhibited [3H]DAMGO (0.5, 2.0 and 8.0 nM) binding with EMAX values of 78.6%, 72.1% and 45.7%, respectively and EC50 values of 955, 1124 and 4527 nM, respectively. Salvinorin A also partially inhibited [3H]diprenorphine (0.02, 0.1 and 0.5 nM) binding with EMAX values of 86.2%, 64%, and 33.6%, respectively and EC50 values of 1231, 866, 3078 nM, respectively. Saturation binding studies with [3H]DAMGO showed that Salvinorin A (10 μM and 30 μM) decreased the mu receptor Bmax and increased the Kd in a dose-dependent, rather than a linear, manner. Saturation binding studies with [3H]diprenorphine showed that Salvinorin A (10 and 40 μM) decreased the mu receptor Bmax and also increased the Kd in a dose-dependent, rather than a linear, manner. Similar findings were observed in rat brain with [3H]DAMGO. Kinetic experiments demonstrated that Salvinorin A altered the dissociation kinetics of both [3H]DAMGO and [3H]diprenorphine binding to mu receptors. Additionally, Salvinorin A acted as an uncompetitive inhibitor of DAMGO-stimulated [35S]-GTP-γ-S binding.
Discussion: Viewed collectively these data support the hypothesis that Salvinorin A allosterically modulates the mu opioid receptor. Certain analogs of Salvinorin A also partially inhibit mu opioid receptors, as well as delta opioid receptors (data not shown). Thus, we anticipate that the Salvinorin A structural template will yield a number of allosteric modulators of opioid receptors. Acknwledgement: This research was supported by the Intramural Research Program of the NIH, NIDA, and National Institute on Drug Abuse grant R01 DA018151-01A2 to Dr. Prisinzano.

5. Effect of Chronic Administration of Fenfluramine and Fluoxetine on Fenfluramine-Induced Increases in Plasma Serotonin in Rats

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Background: Under normal circumstances, plasma serotonin (5-HT) levels are kept low by transporter-mediated uptake of 5-HT into platelets and by 5-HT metabolism. Elevations in plasma 5-HT have been linked to cardiac and pulmonary diseases. Many clinically-relevant drugs, such as anorectics (e.g., fenfluramine) and antidepressants (e.g., fluoxetine) and also abused drugs (e.g. amphetamines) interact with 5-HT transporters (SERT) and could thereby influence plasma 5-HT. Previous work showed that amphetamine analogs administered acutely evoke large dose-dependent increases in plasma 5-HT. The ability of drugs to elevate plasma 5-HT is positively correlated with their potency as 5-HT transporter substrates. Fluoxetine produced small, but significant, increases in plasma 5-HT (Zolkowska, et al., JPET 318:604–610, 2006). In the present work, we examined acute and chronic effects of the 5-HT releaser fenfluramine and the 5-HT uptake blocker fluoxetine on extracellular levels of 5-HT and 5-HIAA in blood obtained from catheterized rats. We also

measured the effect of chronic treatment with these agents on whole blood 5-HT.
Methods: For acute treatments, rats received i.v. injections of saline or test drug (0.3 or 1 mg/kg), and serial blood samples were withdrawn into chilled tubes. For chronic treatments, vehicle or test drug (fluoxetine or fenfluramine) was infused via osmotic minipumps (3 or 10 mg/kg/day) for a period of 14 days. On the last day of infusion, rats received a fenfluramine challenge (1mg/kg iv) and serial blood samples were withdrawn into chilled tubes. In all cases, dialysis probes were placed into blood samples ex vivo and perfused with artificial salt solution; dialysate samples were assayed for 5-HT and 5HIAA using HPLC-ECD.
Results: Baseline plasma 5-HT was <1.0 nM. Acute injections of fenfluramine or fluoxetine elicited dose-dependent elevations in plasma 5-HT. Chronic fenfluramine (3 and 10 mg/kg/day) produced a 1.6- and 2.7-fold increase in baseline plasma 5-HT, respectively, but diminished the ability of acute fenfluramine to elevate dialysate 5-HT by 38% and 57%. Both doses of chronic fenfluramine reduced whole blood 5-HT by ~40%. After chronic fluoxetine, baseline dialysate 5-HT was unchanged and the effects of acute fenfluramine was almost completely eliminated by the 10 mg/kg/day dose. Chronic fluoxetine reduced blood 5-HT by 38% and 57% at the 3 and 10/mg/day doses, respectively.
Discussion: Our results indicate that elevations in baseline plasma 5-HT produced by chronic fenfluramine are far below the μM levels necessary to produce adverse cardiovascular effects. Chronic fenfluramine reduces the ability of acutely administered fenfluramine to further increase plasma 5-HT. Extrapolating to a clinical situation, these data suggest that with daily use, the ability of fenfluramine to increase plasma 5-HT will be reduced. The ability of chronic fluoxetine to block fenfluramine-induced increases in 5-HT confirms the involvement of SERT sites in mediating effects of acute fenfluramine.
This research was supported by the Intramural Research Program of the NIH, NIDA, NIH, DHHS.

6. Subjective, Physiological and Behavioural Effects of Caffeine in High and Low Caffeine Consumers After Sleep Restriction
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Sponsor: Harriet de Wit

Background: Caffeine produces mild psychostimulant effects that are presumed to underlie its widespread use. These effects, which include increased alertness, improved mood and improved psychomotor performance, may be particularly evident in individuals whose mood or performance is impaired, for example by sleep restriction or caffeine withdrawal. Indeed, some have argued that acute effects of caffeine are mainly due to the reversal of the negative effects of caffeine abstinence. This study compared the effects of caffeine in heavy or light habitual caffeine users, in a context of modest sleep restriction.
Methods: Thirty-eight healthy volunteers (17 male, 21 female) were classified by median split on weekly caffeine consumption (mean consumption 1 and 10 cups of coffee per week). Individuals participated in two experimental sessions in which they remained awake without consuming caffeinated beverages from 5 pm to 5 am. At 3:30 am they consumed capsules containing either caffeine (200 mg, Memotrix) or placebo in random order under double blind conditions. Memotrix contains caffeine (200 mg; main active ingredient), white willow bark (50 mg) magnesium oxide (30 mg) and taurine (10 mg). Participants completed subjective effects questionnaires and performed computerized tasks to measure attention before and after consuming the capsules.
Results: Compared to ratings obtained at 5 pm, subjects in both conditions reported more Fatigue, decreased Stimulation, Arousal and High, and impaired performance in a simple attention task at 3 am. There was no evidence of withdrawal at 3 am in the heavier consumers. Memotrix increased stimulant-like effects and increased Want More Drug, compared to placebo. Memotrix also significantly decreased self-reported Fatigue, increased Arousal, and Elation and improved reaction times in the attention tasks. High caffeine consumers had higher heart rate and lower systolic blood pressure than low consumers at 5 pm, but the groups did not differ on any other measures, at 5 pm, 3 am, or after the drug. However, in both conditions heavy habitual caffeine consumers exhibited longer reaction times and reduced accuracy in a task of attention.
Discussion: These findings indicate that caffeine improves mood and performance regardless of level of habitual caffeine use, and thus do not provide support for the withdrawal reversal hypothesis. This research was supported by a grant from Atlas Labs USA and by DA02812.

7. Effects of 24 Hour Smoking Abstinence on Sustained and Event-Related Brain Activity During Continuous Working Memory: Preliminary Results of an fMRI Study
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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Many smokers report difficulty concentrating following abstinence and significant deficits in attention and working memory among abstinent smokers can persist for as long as one month. We hypothesized that these deficits result from decreased brain activity in fronto-parietal attentional and working memory networks; and that abstinence also results in the recruitment of regions involved in effortful processing.
Methods: To evaluate these hypotheses, we measured fMRI-BOLD signal in dependent smokers on two occasions: once after smoking as usual and once following 24 hr abstinence. During scanning, participants completed a continuous working memory task in which a stream of single digits is presented on the screen at a rate of 100/minute. During some blocks, participants were required to press a button after seeing three even or three odd numbers in a row (RVIP); while in other blocks they were required to respond upon seeing the number '0' (Control). Data were analyzed to evaluate the effects of abstinence on both sustained (block level) and transient (event-related) brain activity.
Results: Analyses of preliminary data from the first 8 participants (total n will be 16) indicate RVIP specific (RVIP minus Control) sustained activation in regions associated with the fronto-parietal working memory network. Abstinence decreased RVIP-specific sustained activation in left dorsolateral prefrontal cortex (dlPFC; BA 9/46) while increasing activation in left anterior cingulate gyrus (ACG; BA 32). Event-related responses to correctly identified targets were larger during the RVIP task compared to the Control task in a broad range of frontal cortical regions. Abstinence did not affect responses to targets in the Control task; whereas for the RVIP task, abstinence increased the amplitude of responses to targets in dorsal ACG and posterior cingulate gyrus.
Discussion: These preliminary results support our hypotheses by showing that smoking abstinence leads to a decrease in sustained activation in an area subserving working memory (dlPFC); while leading to an increase in sustained and accuracy-dependent transient activity in an area subserving effortful processing (ACG). These findings will be further discussed in the context of smoking withdrawal and relapse.

8. Association Between Acute Effects of Caffeine and D2 Receptor Gene Polymorphisms in Light, Non-Dependent Caffeine Users
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Background: Caffeine produces mild psychostimulant effects by antagonizing adenosine at A1 and A2a receptors, and perhaps through

interactions with other transmitter systems. Adenosine receptors are co-localized and functionally interact with dopamine receptors in the brain. Thus, functional polymorphisms in the genes for either adenosine or dopamine receptors may affect responses to caffeine. Previously, we reported a significant association between A2a and A1 receptor gene polymorphisms and subjective changes after caffeine. In this analysis, we examined associations between the physiological, behavioural and self-reported subjective effects of caffeine and genetic variations in D2 receptor genes (A77G, –141 Ins/DelC, Taq1B A>G, Taq1D G>A, Intron 6 Ins/DelG, His313His, Exon 8 C>G, and Taq1A G>A).
Methods: Healthy male and female individuals (n=102), who consumed less than 300 mg caffeine per week, ingested capsules containing 0, 50, 150, and 450 mg under double blind conditions in four separate experimental sessions. Subjects completed ratings of subjective effects before and at repeated times after taking the capsules, and cardiovascular measures were obtained at regular intervals. Participants also completed behavioural tasks that measured attention and short-term memory 40 minutes after drug administration.
Results: We found a significant association between D2 receptor gene His313His genotype polymorphism and increases in subjective ratings of Stimulation after caffeine. At the highest dose of caffeine, individuals with the T/T genotype reported feeling less Stimulation than other genotypes. We also found a significant association between the Taq1B and Intron6 Ins/DelG polymorphisms and self-ratings of High after caffeine. For both polymorphisms, only individuals with the G/G genotype exhibited significant increases in High. We found no relationships between D2 genotypes and cardiovascular or behavioural performance measures. There were also no significant interactions between D2 genotypes and A2A genotypes studied previously.
Discussion: These findings suggest that dopamine receptor gene polymorphisms may contribute to subjective responses to caffeine. This research was supported by DA20812.

9. Methylphenidate and Pramipexole Drug Effects in Adolescents and Young Adults with Attention Deficit Hyperactivity Disorder (ADHD) and Nicotine Dependence

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Sponsor: Joseph V. Brady

Background: Mesolimbic dopaminergic system is involved in the pathophysiology of nicotine dependence. However, there is a lack of research examining the dopaminergic systems in adolescents and young adults with ADHD and nicotine dependence. In this study, we examined the neuroendocrine response to a dopaminergic challenge in three groups of adolescents- nicotine dependent, with ADHD, and matched controls.
Methods: Thirty-five participants (15 – 20 yr.) were recruited. Neuroendocrine, mood, and behavioral response to the dopaminergic agents- methylphenidate (10 mg), an indirect dopamine agonist, and pramipexole (0.25 mg), a direct dopamine receptor agonist were examined. Measures of these responses were spontaneous eye-blink rate, plasma prolactin (PRL), growth hormone (GH), and a visual analog mood scale (VAMS).
Results: Adolescents with nicotine dependence had a significantly ($p<0.05$) blunted growth hormone response and greater VAMS "euphoric", as well as "energized" response to methylphenidate as compared to controls. Only nicotine dependent participants had a significantly ($p<0.05$) greater VAMS "energized" response to pramipexole as compared to controls.
Discussion: Adolescents with nicotine dependence may have a blunted dopaminergic activity as compared to controls. Only nicotine dependent adolescents had euphoria and increased energy to methylphenidate. Implications of these results are discussed.

10. Cigarette Smoking Predicts Opioid Abuse Among Pain Patients: Clinical and Research Issues

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Background: Although overall rates of abuse of opioid medications appear low in pain sufferers who are prescribed the medications, abuse and aberrant drug-related behavior is not evenly distributed across all subpopulations. The risk is greatest among young adult males with some combination of the following characteristics: histories of opioid abuse, injection use and/or illicit distribution, heavy alcoholic beverage drinking, other drug abuse and addiction, recent marijuana use, and cigarette smoking. Risk factors for aberrant drug-related behavior on opioid therapy can be assessed using any of a number of new screening instruments developed for this purpose. Some of these new tools rely on cigarette smoking as a predictor of elevated risk of opioid abuse. Given that the prevalence of cigarette smoking is two to three times higher among pain patients than the approximately 20% prevalence among adults in the United States, the use of smoking as risk factor for opioid abuse among pain patients carries the risk of potentially overestimating the risk for many such patients if a better understanding of the nature of the smoking aberrant drug-related behavior relationship is not better understood.
Methods: This presentation will include a literature based review with expert input to explore the potential role of cigarette smoking as a predictive factor the risk of opioid misuse and/or abuse in pain patients. We will also examine whether smoking simply serve as a surrogate marker for substance abuse, which, in turn predicts opioid abuse among pain patients. Or, perhaps smoking stands as an independent predictor of opioid abuse and/or iatrogenic addiction even in patients without histories of any other drug abuse? It will also be important to discover if all cigarette smokers at equally elevated risk regardless of level of smoking, degree of nicotine dependence and/or whether they are former smokers? Is cigarette smoking itself the key variable or would assessment of dependence according to DSM IV or ICD 10 criteria be of greater predictive power? Would treatment of tobacco dependence in a pain patient alter their risk of abusing opioids? Perhaps the deleterious impact of smoking on pain and/or opioid metabolism plays a role in this relationship.
Results: Prelimary analysis (to be completed prior to the meeting) indicates that measures of cigarette smoking are predictive of opioid abuse. Furthermore, it appears that measures associated with higher levels of nicotine dependence show stronger correlations with opioid abuse than measures with lower levels of dependence.
Discussion: Additional questions relate to the public health and the practice of medicine. We accept that as a matter of humanitarian and ethical medicine, persons in pain should not be denied opioid medications if these are indicated, even in the presence of potential risk factors such as cigarette smoking and histories of opioid abuse. Just as efforts are made to detect and minimize side-effects of medication therapy, signs of potentially heightened risk of prescription opioid abuse warrant consideration of efforts to minimize the risk, and detect such events if they occur. This suggests development and application of what the FDA now refers to as Risk Minimization Action Plan (RiskMAP). RiskMAPS can include education of health professionals involved in pain management, patient education, enhanced patient follow-up system to be assured that pain relief is adequate and that problems are not occurring, potential involvement of family members and other caregivers. These findings have implications for assessing risk of opioid abuse among pain patients, the plausible role of tobacco use, research questions, and potential risk management strategies.

11. Caudate-Accumbens Volume Deficit in Cocaine Dependence is Reversible

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Research suggests that brain changes in Cocaine Dependence (CD) evolve during the first several months of abstinence and research from our laboratory has suggested that some of these brain changes are reversible. Withdrawal from CD is characterized by anhedonia and diminished reward sensitivity consistent with reduced dopamine neurotransmission in forebrain regions such as the caudate and accumbens nuclei. We determined the combined volume of the caudate and accumbens nuclei in 46 patients with CD and 17 healthy normal controls using high-resolution MRI. Patients were studied in groups according to their abstinence duration allowing us to test whether abnormalities in volume only existed in the early abstinence period when deficits in dopamine neurotransmission are thought to be greatest.
Methods: Subjects were patients in a therapeutic community rehabilitation program (Daytop Village, Inc.) that closely monitored ongoing abstinence. Patients were recruited according to time from last cocaine use: 15 "brief-abstinence" subjects (BriefAB) reported last binge cocaine use <6 weeks (mean = 2.6 w) prior to testing, 15 "medium-abstinence" subjects (MediumAB) reported last cocaine use 2-10 months (38.4 w) prior to testing and a "long-abstinence" group (LongAB) reported last cocaine use >1 year (92.8 w) prior to testing. Seventeen healthy volunteers were matched for age and parental SES. Forty of the 46 patients and 11 of the 17 controls were male. Patients met diagnostic criteria for CD but had no other substance dependence or Axis I disorder. Controls were free of Axis I diagnoses. Seven of the BriefAB subjects were tested for longitudinal (mean interval = 22.7 w) changes. T_1-weighted scans were acquired at 1.5 T using a 1 mm isotropic voxel. Voxels of intracranial tissues were classified by automatic segmentation and images were realigned prior to volume determinations. Parcellation of the combined caudate-accumbens nuclear complex (CANC) was informed by published methods. The caudate tail was excluded from measurements. Absolute volumes were normalized to total intracranial volume and the resulting relative volumes were analyzed using *t*-tests.
Results: Subjects were well matched on demographic variables. Number of years of cocaine use was similar in patient groups and averaged ~10 years. Mean relative CANC volume in BriefAB was significantly lower than that in controls on the right (P=.03) and marginally lower on the left (P=.07). The volume differences were 9.6 and 8.1% on the right and left respectively. MediumAB and LongAB groups did not show differences in CANC compared with controls. Mean relative CANC volume in BriefAB also was significantly lower than that in LongAB subjects on the right (P=.05) and were marginally lower on the left (P=.09). When only male patients were compared, volume in BriefAB was significantly lower that that in LongAB bilaterally. Comparison of the time 1 and time 2 scans from seven longitudinally tested male subjects showed significant increase (P=.05) in CANC volume over time on paired *t*-test on the right side only.
Discussion: In contrast to the data of Jacobsen et al. (2001), we found CANC volume reduction in chronic cocaine users meeting criteria for CD. Our data also show that CANC volume deficits were fully reversible. Our findings are opposite to enlarged caudate volumes in schizophrenia patients treated chronically with conventional antipsychotic medications. These findings are consistent with the idea that CANC volume changes are sensitive to dopamine effects. It will be of interest to see if CANC volume changes are related to dopamine-sensitive deficits brain functions such as reward sensitivity in CD. The right hemisphere lateralization of these effects adds to a growing literature relating right hemisphere changes in substance use disorders.

12. Excessive Alcohol Consumption Sensitizes Accumbens Glutamate Transmission: Link to Homer Proteins and Kinase Activation

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Background: The advent of the Scheduled High Alcohol Consumption (SHAC) partial animal model of alcoholism has provided a simple tool with which to investigate the neurobiology of repeated bouts of high alcohol intake or "binging" in laboratory rodents. A bout of SHAC elevates blood alcohol levels (BACs) above 100 mg%, thus, this model enables investigation into the neuroadaptations produced by repeated intake of pharmacologically significant concentrations of alcohol. Alcohol is a drug of abuse well-characterized to affect dopamine and glutamate transmission in the brain and Homer2 proteins appear to be critical regulators of alcohol-induced changes in these neurotransmitter systems. The present study employed the SHAC model to ascertain the effects of repeated bouts of binge-like drinking upon extracellular levels of dopamine and glutamate in the nucleus accumbens (NAC) and upon the expression of Homer proteins and their related glutamate receptors within this brain region.
Methods: C57BL/6J mice were trained to drink a 5% alcohol solution during a 40-min period, every third day for a total of 6 days (SHAC6). On the 6th alcohol presentation, in vivo microdialysis was conducted in the NAC, before, during and after the 40-min drinking session. Two control groups were included. One control drank water throughout, while the other control drank 5% alcohol during the microdialysis session (SHAC1). In a parallel experiment, immunoblotting for Homer2, Homer1b/c, the mGluR5 and mGluR1 subtypes of Group 1 mGluRs, the NR2a and NR2b subunits of the NMDA receptor, as well as total and activated PI3K and PKC epsilon was conducted on NAC tissue from SHAC6 and water drinking mice.
Results: SHAC mice consumed approximately 1.5 g/kg alcohol during the microdialysis session and intake during microdialysis was equivalent between SHAC1 and SHAC6 mice. Compared to water controls, alcohol intake elevated NAC dopamine to a similar extent in SHAC1 and SHAC6 mice. While neither the water controls nor the SHAC1 mice exhibited a rise in NAC glutamate during fluid consumption, SHAC6 mice exhibited a large increase in NAC glutamate levels that persisted following alcohol consumption. Immunoblotting on NAC tissue from SHAC6 mice revealed a selective increase in Homer2 expression that was accompanied by an elevation in NR2 subunits, the activated forms of PI3K and PKC epsilon, but not in either Group 1 mGluR subtype.
Discussion: These data provide the first evidence that a history of "binge-like" drinking sensitizes glutamate neurotransmission within the NAC. Our earlier behavioral genetic studies demonstrated a critical role for Homer2 in regulating alcohol intake, alcohol-induced neuroplasticity within the NAC and glutamate receptor function/expression. These findings are consistent with the present observations that SHAC increases the total protein expression of both Homer2 and NR2 subunits in the NAC and further implicate Homer2 as an important regulator of NMDA receptor expression in vivo. While SHAC did not influence the total expression of Group 1 mGluRs, it up-regulated the activation of down-stream targets of these receptors. This suggests that SHAC induces cellular adaptations in excitatory glutamate receptor signaling within the NAC, that likely contributes to the development of glutamate sensitization, a hypothesized putative mediator of excessive alcohol drinking behavior. Supported by funds from NIAAA (AA15351 and AA013517-INIA West) to KKS.

13. Atomoxetine Treatment of Adults with ADHD and Comorbid Alcohol Abuse Disorder

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Background: Patients with attention-deficit/hyperactivity disorder (ADHD) are known to have higher rates of alcohol and drug abuse than control populations, a fact that by itself markedly increases risks for poor outcomes (Wilens TE, et al., 1995, Psychiatr Serv 46(8):761-763, 765). This study tested the hypothesis that atomoxetine (ATX) is superior to placebo (PBO) in the treatment of ADHD symptoms and prevention of relapse of alcohol abuse in adult patients with both ADHD and comorbid alcohol abuse disorder who are recently abstinent.
Methods: Participants were adults who met full DSM-IV-TR criteria for both ADHD (including a historical diagnosis of childhood ADHD) and alcohol abuse disorder. Patients had to be abstinent from alcohol 4-30 days to enter the study. Participants were randomly assigned to receive ATX (25-100 mg daily as either a single daily dose or a divided twice daily dose) or PBO for approximately 12 weeks. ADHD symptoms were assessed using the ADHD Investigator Symptom Rating Scale (AISRS) Total score. Time to relapse of alcohol abuse (4 standard alcoholic drinks for females or 5 standard alcoholic drinks for males within 24 hours, or at least 3 standard alcoholic drinks/day for at least 1 week) was defined as the amount of time (in days) from first dose of study medication to first occurrence of relapse using the Timeline Follow-back method for determining daily alcohol amounts (Sobell LC and Sobell MB, 1992, In: Measuring Alcohol Consumption. Totowa (NJ): The Humana Press Inc. p 41-72) and was analyzed using a 2-sided log-rank test based on Kaplan-Meier estimates. Cumulative heavy drinking events over time was measured post hoc with a recurrent event analysis using a stratified Andersen-Gill recurrent-event Cox model (Wang SJ, et al., 2002, Alcoholism: Clinical & Experimental Research 26(12):1803-9). This analysis, though not originally planned, may be considered more appropriate (e.g., Garbutt JC, et al., 2005, JAMA 293(13):1617-25).
Results: One hundred forty-seven (147) patients were randomly assigned to receive ATX (n=72) or PBO (n=75). ADHD symptoms were significantly improved in the ATX cohort as compared with PBO (AISRS total score mean [SD], ATX: -13.63 [11.35], P<.001; PBO: -8.31 [11.34], P<.001, Difference: P=.007). While analysis of time to relapse showed no significant differences between treatment arms (P=.934), the recurrent event analysis demonstrated that atomoxetine significantly reduced the cumulative heavy drinking rate by approximately 24% compared to placebo (hazard ratio=0.761, P=.0380). There were no serious adverse events, and the adverse event profile was similar to what has been demonstrated in previous studies.
Discussion: This 3-month double-blind placebo-controlled study of adult ADHD patients with comorbid alcohol abuse demonstrates robust effects in reducing ADHD symptom severity and suggests a positive effect for ATX in reducing cumulative heavy drinking events over time.

14. A Pilot Study of the Pharmacological Effects of Alcohol and Naltrexone on Craving for Cigarettes

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Sponsor: Robert Swift

Background: Research studies have demonstrated that the urge to smoke cigarettes increases when smokers consume alcohol. Naltrexone has been shown to reduce the urge to drink and to attenuate the reinforcing value of nicotine. Although naltrexone has been widely researched in the context of drinking and smoking behaviors, little is known about the combined effects of naltrexone on craving for alcohol and cigarettes. The present study used a within subjects double-blind placebo-controlled design to: (1) examine the effects of alcohol on urge to smoke cigarettes; (2) test the effects of naltrexone on urge to smoke cigarettes during alcohol intoxication; (3) compare craving for alcohol and cigarettes across rising levels of Breath Alcohol Concentration (BAC).
Methods: Participants (N=10) were light smokers who were also heavy drinkers. Participants completed two intravenous alcohol challenge sessions, one after taking naltrexone (50mg) for three days, and one after taking a placebo for three days. During each session, participants received alcohol through an IV and reported on their craving for alcohol and cigarettes at each target BAC, .02, .04, and .06.
Results: A series of repeated measures mixed effect models revealed a significant effect of BAC on urge to smoke, such that the urge for cigarettes increased across rising levels of BAC ($\chi 2(1)=7.89$, $p<.01$). There was also a significant effect of medication on urge to drink ($\chi 2(1)=12.63$, $p<.001$), such that participants reported lower alcohol-induced urge to smoke in the naltrexone condition, as compared to placebo. Analysis of the relationship between urge to drink and urge to smoke revealed a positive association between craving for alcohol and craving for cigarettes ($\chi 2(1)=6.20$, $p<.05$), as well as a significant Urge X BAC interaction ($\chi 2(1)=5.48$, $p<.05$) suggesting that the relationship was strongest at lower levels of BAC.
Discussion: Taken together, the present findings demonstrate that the pharmacological effects of alcohol induce craving for cigarettes, among light smokers, even in the absence of alcohol cues. This pilot study also provides preliminary evidence that naltrexone may reduce the urge to smoke during alcohol intake. Lastly, the positive relationship between the urge to drink and the urge to smoke suggests that common mechanisms may be underlying craving for both substances. Study limitations include the small sample size and absence of a placebo alcohol condition. Research supported by NIAAA grant F31 AA14847 to LAR.

15. Chronic Recordings of Rat Orbitofrontal Cortical Neurons During Intravenous Cocaine Self-Administration: Findings and Comparisons to Neuro-Imaging Studies of Humans

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Sponsor: George Woody

Background: Neuro-imaging studies of cocaine-addicted humans have demonstrated cue- and drug-induced activation of the orbitofrontal cortex (OFC), as well as abnormalities in basal function relative to drug-naive humans. These and other findings have implicated OFC in the development of addiction to cocaine. Despite the evidence of the OFC's involvement in cocaine addiction, there are gaps in our understanding of the mechanisms that mediate that role. Imaging studies provide an indirect measure of neural activity and are limited to sampling with relatively coarse spatial and temporal resolution. Additionally, imaging studies of drugs such as cocaine are limited to investigating subjects with an extended history of cocaine exposure so that it is difficult to ask certain questions regarding the development of drug addiction. Some of these questions are subject to investigation using chronic electrophysiological recordings conducted during sessions in which rats self-administer drug.
Methods: In the present study we recorded the activity of single OFC neurons during sessions in which rats self-administered cocaine (maximum of 3 hrs per day, 0.75 mg/kg/infusion). Recordings were conducted on the 1st day of intravenous self-administration, and during two sessions that occurred between the 10th – 14th day of self-administration.
Results: During both the early and later sessions, the population of OFC neurons (> 150 during each session) showed an overall increase in average firing rate during the self-administration session relative to drug-free periods. The average basal firing rate (i.e., firing during a

pre-session baseline period), and firing during the self-administration session, following a 24hr withdrawal period, were significantly elevated relative to those recorded on the first day of cocaine self-administration. Additionally, the population of neurons showed a pattern of modulation between successive self-infusions that was inversely related to calculated drug level. Additional analysis showed that the responses of individual neurons were heterogenous, with some neurons showing a strong increase in firing during the self-administration session and others showing a decrease. Individual neurons also showed rapid phasic responses during the seconds that bracketed self-infusion behavior and cue presentation. Interestingly, there was evidence of a relationship between the overall session change in firing and the amplitude of the phasic firing, such that the changes in overall firing rate associated with drug exposure amplified the phasic responses time-locked to the self-infusion behavior.
Discussion: The relationship between average (tonic) firing and phasic firing patterns during drug exposure is consistent with that observed during previous recordings of accumbal neurons during cocaine self-administration, and with the hypothesis that cocaine-induced acute (and chronic) drug-induced changes in basal activity may increase the signal-to-background ratio of neural signals related to drug seeking and may thereby contribute to drug-directed behavior (Peoples and Cavanaugh, 2003, J Neurophysiology 90:993-1010). Overall, the population responses of the OFC neurons are strikingly similar to patterns of metabolic activity in relevant human neuro-imaging studies. However, the findings also provide new insights into mechanisms that may be relevant to drug addiction, and that are not readily subject to investigation in neuro-imaging studies of humans. The present study provides evidence that chronic recording studies in the animal model of drug self-administration may provide a useful translational tool for characterizing novel neuronal mechanisms that contribute to cocaine addiction.

16. Clarity of State Guidance on Infection-Related Health Services in Substance Abuse Treatment Programs

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Background: The Infections and Substance Abuse Study (NIDA CTN-0012) examined associations between services provided for HIV/AIDS, hepatitis C viral infection (HCV), and sexually transmitted infection (STI) at substance abuse treatment programs in the National Drug Abuse Treatment Clinical Trials Network (NIDA CTN), and the states within which they are located. This report specifically looks at the relationships between clarity of state policy, regulations and guidelines as viewed by clinicians, and the availability of eight infection-related services for all three disease groups.
Methods: Data for this report was derived from two surveys: one for state health and substance abuse department administrators, and one for substance abuse treatment program clinicians. The surveys included questions dealing with eight infection-related services: provider education, patient education, risk assessment, counseling, medical history and physical exam, biological testing, treatment and monitoring.
Results: Administrators of state substance abuse and/or health departments from 48 states and the District of Columbia participated. Surveys were also obtained from 1723 clinicians (78%) within the NIDA CTN: 251 medical experts (15%), 522 non-medical experts (30%), 115 medical non-experts (7%) and 831 non-medical non-experts (48%). With few exceptions, clinician assessment of clarity of state policies, regulations and guidelines for the eight targeted services is generally about 50% or less for all three infection groups. Six of eight services were provided by a similar percentage of programs, regardless of state mandates. Two services (treatment and monitoring) were provided by a substantially higher percentage of sites where there were state policies, regulations or guidelines.
Discussion: This information suggests that state policies, regulations or guidelines are often not clearly understood by clinicians, and even where they exist, they are not sufficient to assure "best practices" in treating these epidemic infections.

17. Higher Suicidality in Patients with Comorbid Major Depression and Alcohol Use Disorders

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Sponsor: David A. Brent

Background: Alcohol abuse and dependence (alcohol use disorders; AUD) and major depressive disorder (MDD) are important causes of morbidity and mortality in the Western countries. AUD and MDD are often comorbid. Studies suggest that MDD subjects with comorbid AUD have more chronic impairment and suicidal behavior than individuals with either diagnosis alone. The reason for higher rate of suicide and suicide attempts in comorbid subjects is uncertain. We explored clinical characteristics that may be associated with this increased suicidality in a large sample of MDD patients with and without comorbid AUD.
Methods: In all, 503 subjects who met DSM-IV criteria for MDD were included in the study. Three hundred sixteen MDD individuals did not have a history of any alcohol or substance use disorders (MDD only) and 187 depressed subjects had comorbid AUD (MDD/AUD). Demographic and clinical parameters including Aggression History Scale (Brown-Goodwin, revised), Barratt Impulsivity Scale, Buss-Durke Hostility Scale, Scale for Suicide Ideation, Reasons for Living Scale, and St. Paul Ramsey Life Events Scale scores and a lifetime history of all suicide attempts were assessed and recorded.
Results: There were more males than females in the MDD/AUD group compared to the MDD only group (chi-square=9.0, df=1, p=0.003). MDD/AUD subjects were younger at the time of entry to the study (t=3.2, df=501, p=0.001) and reported more major depressive episodes (t= -2.9; df=462, p=0.004) and recent life events (t= -3.3, df=262, p=0.001) compared to MDD only patients. Both before and after controlling for gender and age MDD/AUD subjects had higher lifetime aggression (before: t= -11.4, df=444, p<0.001; after: df=3,442, F=55.9, p<0.001), impulsivity (before: t= -4,7, df=401, p<0.001; after: df=3,399, F=10.1, p<0.001), hostility (before: t= -5.7, df=416, p<0.001; after: df=3,414, F=19.3, p<0.001), were more likely to report tobacco smoking (before: chi-square=42.1, df=1, p<0.001; after: df=3,473, F=17.3, p<0.001) and past suicide attempts (before: chi-square =12.4, df=1, p=0.001; after: df=3,499, F=9.9, p<0.001) compared to the MDD only group. Controlling for age and gender MDD/AUD patients had higher current suicide ideation (df=3,287, F=13.5, p<0.001) and lower reasons of living (df=3,273, F=3.2, p=0.03) scale scores compared to the MDD only group. Both before and after controlling for gender MDD/AUD patients were younger at the time of their first psychiatric hospitalization (before: t=3.3, df=341, p=0.001; after: df=2,340, F=5.6, p=0.004) compared to MDD only patients. Controlling for gender MDD/AUD subjects were younger at the time of their first major depressive episode (df=2,467, F=6.2, p=0.002) compared to MDD only subjects.
Discussion: MDD patients with comorbid alcohol use disorders report more suicidal behavior than MDD patients without comorbid alcohol use disorders. This increased suicidality may be related to higher aggression, impulsivity, and hostility in the MDD/AUD group which is consistent with the stress-diathesis model of suicidal behavior. Chronic alcohol intake may lead to a state of lowered central serotonergic functioning characterized by a propensity towards disinhibited behavior, thus increasing the potential for aggressive, impulsive, and hostile behavior. Our findings suggest that in addition to obtaining a history of depression and suicidal behavior, clinicians should assess comorbidity with AUD and personality traits such as aggression

and impulsivity. This may help identify patients at higher risk for suicidal behavior.

18. Brain Region-Specific Alterations of Endocannabinoid Levels Induced by Positive and Negative Affective States of Ethanol-Dependence

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Sponsor: Travel Awardee, NIMH, 2006

Background: Chronic ethanol (EtOH) exposure can dysregulate the neural mechanisms mediating positive reinforcement and the development of dependence is characterized by the presence of negative affective states such as anxiety during EtOH abstinence. However, our understanding of the range of mechanisms involved in these processes is incomplete. In this regard, recent evidence has demonstrated that the endocannabinoid (eCB) system is involved in modulating the motivational effects produced by EtOH. We have observed that EtOH self-administration increases extracellular eCB levels in the nucleus accumbens (NAC) and that EtOH intake is sensitive to alterations in eCB signaling. Furthermore, in light of evidence that eCBs modulate anxiety-like behavior it is possible they play some role in the anxiety-like states and activation of stress circuitry present during acute EtOH withdrawal.
Methods: In the present set of experiments, we investigate alterations in eCB signaling induced by chronic EtOH exposure and during acute abstinence using *in vivo* microdialysis.
Results: We have found that baseline dialysate anandamide (AEA) and 2-arachidonoylglycerol (2-AG) levels are significantly altered in the NAC of rats given chronic EtOH exposure via a liquid diet procedure as compared with controls. In addition, non-contingent EtOH administration (2 g/kg, i.p.) significantly increases NAC dialysate 2-AG levels in EtOH-naïve controls, and this effect is enhanced and prolonged in chronic EtOH-exposed animals. In addition, we have found that both interstitial AEA and 2-AG are transiently suppressed in the central nucleus of the amygdala (CeA) during an acute 12h period of abstinence from chronic EtOH liquid diet exposure.
Discussion: These findings provide *in vivo* evidence of altered baseline eCB levels and eCB responsivity to EtOH challenge following a regimen of chronic EtOH administration and during acute withdrawal, and provide additional evidence implicating the eCB system in the mediation of EtOH-induced behaviors.

19. Behavioral and Neurochemical Effects of Repeated Cocaine Administration in Adolescent and Adult Mice

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Background: Adolescence has been hypothesized to be a period of vulnerability whereby exposure to drugs of abuse can cause persistent changes in the brain, which can in turn increase the likelihood of the development of substance abuse problems in adulthood. Previous studies examining potential differences in behavioral and neurochemical responses to cocaine in adolescent versus adult rodents have produced conflicting results, with some studies demonstrating that adolescent rodents are more sensitive to the effects of psychostimulants than their adult counterparts, whereas others have shown that adolescents are hyposensitive. The present study sought to clarify this issue by examining locomotor responses to repeated cocaine administration in adolescent and adult mice and alterations in extracellular levels of dopamine and glutamate in the nucleus accumbens (NAc) in response to a subsequent cocaine challenge.
Methods: Male adolescent (P30) and adult (P60) DBA/2J mice were used as subjects. Mice were given once daily injections of either saline or cocaine (10 mg/kg i.p.) for 9 consecutive days. Locomotor activity was monitored following drug administration for 15 min on days 1, 2, and 9. One week following the last drug administration, animals were implanted with microdialysis probes into the NAc to examine changes in extracellular levels dopamine and glutamate following an acute challenge with cocaine (10 mg/kg i.p.).
Results: Adolescent and adult mice displayed similar locomotor responses to cocaine on day 1 of treatment (adolescents 6014 +/- 616, adults 7048 +/- 659 cm traveled). Both groups of mice displayed sensitization to the locomotor stimulant effects of cocaine, but adolescents displayed higher levels of locomotor activity (10928 +/- 551 cm traveled) compared to adults (9346 +/- 579 cm traveled) on day 9 of cocaine treatment. Saline-treated mice displayed no evidence of increased locomotor activity on any day of treatment. Microdialysis procedures revealed that cocaine-sensitized adult mice displayed greater peak increases in extracellular dopamine in the NAc (389 +/- 39% above baseline) in response to a subsequent cocaine challenge as compared to saline-treated adult mice (280 +/- 43% above baseline), cocaine-sensitized adolescent mice (188 +/- 14% above baseline) and saline-treated adolescent mice (153 +/- 17% above baseline). In addition, area under the curve analysis revealed a greater overall increase in extracellular dopamine during the 2 hr post-injection period in cocaine-sensitized adult mice (1212 +/- 79, arbitrary units) as compared with saline-treated adult mice (744 +/- 172), cocaine-sensitized adolescent mice (731 +/- 65) and saline-treated adolescent mice (590 +/- 86). Analysis of changes in extracellular glutamate is currently ongoing.
Discussion: Our data suggest that adolescent mice are hypersensitive to the locomotor stimulant effects of repeated cocaine administration as compared to adult mice. However, there appears to be a dissociation between the locomotor effects of repeated cocaine exposure and subsequent cocaine-induced alterations in extracellular dopamine in the NAc, with adolescent mice displaying greater locomotor responses to repeated cocaine compared with adults, but reduced peak and overall increases in dopamine in the NAc in response to cocaine challenge. Future studies are needed to determine how these behavioral and neurochemical correlates of repeated cocaine exposure in adolescent vs. adult animals predict subsequent vulnerability to cocaine addiction. *All procedures performed within NIH Guidelines; Supported by the Center for Drug and Alcohol Programs at the Medical University of South Carolina, and by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

20. Chronic Intermittent Ethanol Treatment Increases NMDA Receptor Subunits Surface Expression in Cultured Cortical Neurons

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Background: A chronic intermittent ethanol (CIE) exposure regimen consists of repeated episodes of ethanol intoxication and withdrawal. In vivo animal model, CIE treatment has been reported to result in a significant enhancement of NMDA receptor-mediated synaptic response. Trafficking of NMDA receptor is emerging a key regulatory mechanism that underlies the channel function. NMDA receptors in cortical neurons are composed of the requisite NR1 subunit and the NR2A and/or NR2B subunit. For NR2 subunits, NR2B subunit-containing receptors are thought to be more sensitive to ethanol.
Methods: Cultured cortical neurons were exposed to 75 mM ethanol for 14 h followed by 10 h withdrawal, repeated 5 times and followed by 2 or 5 days withdrawal. Surface expressed NR1 and NR2B and their endocytosis were measured by biotinylation and Western blots.Quantification of immunohistochemistry was also used in this study.
Results: In the present study we examined the effects of CIE on the surface expression of NMDA receptor NR1 and NR2B subunits. The results demonstrated that CIE significantly increased NR1 and NR2B

surface expression after 5 days treatment. CIE treatment did not reduce their endocytosis. Quantification of immunohistochemistry confirmed CIE-induced increase in both the total number of NR1 and NR2B subunit clusters and their targeting to synaptic sites. Importantly, the increased expression in the plasma membrane persisted to 5 days after withdrawal.
Discussion: In recent years there had been increasing evidence demonstrating that NMDA receptor surface expression is regulated by different ways including trafficking between intracellular pools and plasma membrane pools and the lateral movement of surface NMDA receptors through the membrane between synaptic and extrasynaptic sites. Also, the trafficking of NMDA receptor was revealed under a variety of physiological and pathological conditions, therefore suggesting an important role in regulating NMDA transmission and brain function. The present results suggest that CIE-induced changes may contribute to the ethanol-induced enhanced neurotoxicity and ethanol dependence.

21. Cocaine Self-Administration in Humans: A PET Study of Serotonin-Dopamine Interactions

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Sponsor: Paul Vezina

Background: The most common route of cocaine self-administration is intra-nasal, but it remains unknown whether this increases striatal dopamine transmission. Regional differences in this response might be important. Dopamine transmission within the limbic striatum has been proposed to enhance the appetitive salience of reward-related stimuli and susceptibility to drug 'wanting',[1] while dopamine release in the dorsal striatum is thought to be more closely related to habit-like behavior.[2] Serotonin transmission, in comparison, might affect each of these responses by modulating dopamine release[3] and impulse-control.[4] In the present study we (1) measured whether intra-nasal cocaine self-administration increases synaptic dopamine levels in humans, and (2) tested whether the dopamine and drug craving responses would be altered by acute tryptophan depletion (ATD), a method for transiently decreasing serotonin synthesis.
Methods: Eight non-dependent cocaine users (6 men, 2 women; 23.5±3.2 y.o.; cocaine use in the last year, 18.6±6.1 occasions) underwent Positron Emission Tomography scans with [^{11}C]raclopride following the ingestion of (1) cocaine hydrochloride (1.0 mg/kg, intranasal) plus ATD, (2) cocaine plus a nutritionally balanced mixture (BAL), and (3) placebo powder (100mg lactose) plus BAL. Statistical parametric maps of changes in [^{11}C]raclopride binding potentials (BP) were generated. Regions of interest were manually drawn in limbic, associative, and sensori-motor striatum on each individual's MRI, and partial volume corrected BP values were extracted. Self-report measures of drug craving were assessed throughout.
Results: Intra-nasal cocaine, compared to placebo, increased drug craving ($p \leq 0.02$) and decreased [^{11}C]raclopride BP (peak $t = 6.3$) with statistically significant effects occurring preferentially in the limbic ventral striatum ($p \leq 0.02$). The effect of ATD + cocaine was significantly greater than cocaine alone in the dorsal striatum only (peak $t = 4.9$), and cocaine's effect on craving was left unaltered.
Discussion: To our knowledge this study provides the first evidence that (i) synaptic dopamine levels are increased in human striatum by intra-nasal cocaine, and (ii) that low serotonin transmission might augment this response in the sensorimotor dorsal striatum. Since reducing dopamine synthesis decreases cocaine craving,[5] these findings support the hypothesis that drug-induced increases in limbic dopamine transmission contribute to the production of craving states. Low serotonin-related increases in dopamine release within the dorsal striatum, in comparison, might be more important for stimulus-response habit behavior, and susceptibility to dysregulated compulsive drug-seeking. REFERENCES (1) Robinson & Berridge *Brain Res Brain Res Rev* 1993;18:247 (2) Ito et al. *J Neuroscience* 2002;22:6247 (3) Gervais & Rouillard *Synapse* 2000;35:281 (4) Virkkunen et al. *Arch Gen Psychiatry* 1994;51:20 (5) Leyton et al. *Behav Neuroscience* 2005;119:1619

22. Human Laboratory Model of Marijuana Relapse: Effects of Lofexidine and THC

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Sponsor: Richard W. Foltin

Background: Only a small percentage of individuals seeking treatment for their marijuana use is able to achieve sustained abstinence. The objective of this study was to determine if lofexidine, an α2-receptor agonist, and THC, a cannabinoid agonist, given alone and in combination, decreased symptoms of marijuana withdrawal and relapse, defined as a return to marijuana use after a period of abstinence.
Methods: Male nontreatment-seeking volunteers (n=8), who reported smoking 12 marijuana cigarettes/day, 7 days week, were maintained on each of four medication conditions for 8 inpatient days: placebo, lofexidine (2.4 mg/day), oral THC (60 mg/day), and oral THC (60 mg/day) combined with lofexidine (2.4 mg/day). During the inpatient phases, participants had the opportunity to self-administer placebo marijuana for 3 days (marijuana withdrawal phase) and then to self-administer active marijuana for 4 days (marijuana relapse phase). Relapse to marijuana use was costly: participants had to pay for self-administered marijuana using actual study earnings. Each inpatient phase was separated by an 8-11 day outpatient washout phase.
Results: THC alone decreased marijuana withdrawal symptoms (chills, anorexia, subjective ratings of disrupted sleep), but did not decrease marijuana relapse. Lofexidine alone decreased some withdrawal symptoms (chills) but was sedating and did not decrease marijuana relapse. Lofexidine and THC together decreased both a range of marijuana withdrawal symptoms (marijuana craving, chills, restlessness, subjective and objective measures of sleep disruption) and decreased marijuana relapse.
Discussion: The combination of lofexidine and THC decreased relapse to marijuana use in abstinent, heavy marijuana smokers. This medication combination also improved sleep and decreased marijuana craving. These data suggest the combination of lofexidine and THC shows promise as a treatment for marijuana dependence.

23. Analgesic Actions of Fentanyl and Hydrocodone in Rats Treated with Extended-Release Naltrexone

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Naltrexone antagonizes opioid-mediated analgesia via opioid-receptor blockade. Yet there is little information available regarding the feasibility of overriding opioid-receptor blockade with agents commonly used for pain management. The objectives of the present study were to determine whether, and at what doses, fentanyl or hydrocodone can elicit analgesia-like effects in extended-release naltrexone (XR-NTX)-pretreated rats, and if such doses would produce changes in respiration rate or locomotor activity.
Methods: Rats were treated (sc) with placebo or XR-NTX — a formulation containing naltrexone (50 mg/kg) incorporated in microspheres composed of polylactide-co-glycolide. Using the hot-plate test (a pain/analgesia paradigm), on days 5, 20 and 39 after XR-NTX administration, rats were treated with fentanyl citrate (sc) or hydrocodone bitartrate (ip) to determine the dose required to produce maximum response latency (MRL; 60 s) to lick either hind paw. The

day following each test, rats were treated again with fentanyl or hydrocodone to determine their effects on respiration rate (using Buxco whole-body plethysmograph with conscious unrestrained rats) and locomotor activity (using automated open field chambers). Blood samples were collected following testing on each day to determine naltrexone levels using liquid chromatography mass spectrometry (LCMS).
Results: Mean plasma concentrations of naltrexone were 6.1, 3.5 and <0.5 ng/mL on days 5, 20 and 39, respectively. In placebo-treated rats, a dose of 0.1 mg/kg fentanyl produced MRL and depressed respiration rate by 45%. In XR-NTX-treated rats, doses required to produce MRL were 2.0 mg/kg (on days 5 and 20) and 0.5 mg/kg (day 39). The higher doses of fentanyl required to overcome opioid blockade did not result in further depression of respiration when compared to that observed in placebo-treated rats. In placebo-treated rats, a dose of 8 mg/kg hydrocodone produced MRL. In XR-NTX-treated rats, a dose of 80 mg/kg hydrocodone produced 69%, 80% and 100% of MRL on days 5, 20 and 39, respectively. In addition, rats treated with 24 mg/kg hydrocodone on day 39 produced 75% of MRL. XR-NTX-treated rats showed no change in respiration rate at the higher doses of hydrocodone tested. Neither fentanyl nor hydrocodone altered locomotor activity at any of the doses tested in placebo- or XR-NTX-treated rats.
Discussion: In rats pretreated with XR-NTX, fentanyl and hydrocodone both produced analgesia-like effects at doses that did not cause additional decrements in respiration rate or open-field locomotor activity. These results suggest it is feasible to produce analgesia during treatment with XR-NTX by overriding opioid-receptor blockade with higher doses of opioid agonists. These studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

24. Neurochemical Profile of Methamphetamine Determined with Proton Magnetic Resonance Spectroscopy: Relationship to Monoamine Neurotoxicity

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Background: In experimental animals, repeated exposure to stimulants such as methamphetamine (METH) or 3,4-methylenedioxymethamphetamine (MDMA) produces a long-lasting neurotoxicity to monoamine-containing neurons. It remains to be determined if similar damage occurs in human substance abusers. Non-invasive assessment of neurochemical status with proton magnetic resonance spectroscopy (^{1}H-MRS) offers substantial promise to assess potential stimulant-induced neurotoxicity in humans exposed repeatedly to amphetamines. Although monoamine content in brain cannot be measured with ^{1}H-MRS, compounds such as N-acetylaspartate (NAA), which is found only in neuronal mitochondria and thought to represent a marker of neuronal density, is readily identified in clinical scans even at low field strength.
Methods: Therefore, using high resolution magic angle spinning ^{1}H-MRS at 11.7 Tesla, we determined the profile of MR-visible neurochemicals in intact discrete tissue punches (2x2mm, 3 mg) obtained from rats exposed to METH (5.0-7.5 mg/kg ip x4 q2h). Spectra were acquired with a standard CPMG pulse sequence and data were automatically quantified with the LCModel. Monoamine (dopamine, serotonin, metabolites) content in contralateral tissue punches from cortical, striatal, and midbrain subregions were evaluated with reversed-phase HPLC-EC analysis of acid extracts.
Results: When assessed 2.5 hrs after the last METH dose, MR-visible glutamate (GLU) was significantly ($p<0.05$) increased in the accumbens, anterior striatum, CA1 and VTA whereas glutamine (GLN) was increased in the anterior cingulate cortex, striatum and VTA. In the same animals, accumbal DA was reduced 27% ($p<0.05$) in the contralateral punch. At this early stage, changes in cortical GLN/GLU were correlated with monoamine levels. Seven days after the last METH dose, lesion status was evidenced by accumbal DA at 58% , HVA at 63%, and DOPAC/DA at 181% (all $p<0.05$, N=3) of saline treated controls (N=8). The ^{1}H-MRS profile of the contralateral accumbens was unremarkable; notably, levels of NAA, GLU, GLN, inositol, and GABA did not differ from controls.
Discussion: The acute effects of METH on the ^{1}H-MRS neurochemical profile are consistent with a hyperglutamatergic status initiating a cascade that is ultimately neurotoxic to monoamine neurons. As such, the results suggest that ^{1}H-MRS may be useful in the early detection of glutamate dependent pathologies. In animals allowed to recover for 7 days, monoamine neurotoxicity was not accompanied by a decrease in NAA or other relevant MR-visible neurochemicals in the nucleus accumbens. The lack of an effect on NAA may reflect an anatomical specificity, the relative density of DA neurons in the region of interest, and/or the extent of neuronal damage. Thus, the use of NAA as a marker for stimulant-induced neurotoxicity in humans should be interpreted with caution. On-going ^{1}H-MRS/HPLC analyses of cortical and midbrain regions, as well as a comparison to the effects of MDMA, will provide additional insight into the application of neuroimaging spectroscopy to neurotoxic events in humans. Support: NIDA R01-16736 (MPG) & Joe Young Research Fund.

25. White Matter Development and Myelination in the Human Neonate: Relationship of Structural MR and Diffusion Tensor Properties in the Corpus Callosum and Cortico-Spinal Tract

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Background: Brain development in the early postnatal period is perhaps the most dynamic phase of brain development. In the cortex, there is rapid elaboration of new synapses in the first years of life. Cortical gray matter volumes increase rapidly in the first weeks after birth, with occipital/parietal regions growing significantly faster than prefrontal regions (Gilmore et al., 2006), consistent with the regional differences in synapse development. Myelination of white matter also proceeds rapidly in the first years after birth, with the overall pattern of adult myelination completed by the end of the second year of life.
Methods: To better understand the early postnatal development of white matter in normal infants, we obtained high resolution structural and DTI scans in the first weeks after birth in 49 neonates on a 3T scanner, and utilized a novel quantitative analysis of complex regions of interest (Corouge et al., 2006) along with co-registration of DTI images with structural images to study the development of diffusion properties in the corpus callosum and the cortico-spinal tracts. We also studies the relationship of diffusion properties to T1 and T2 properties in these developing white matter tracts.
Results: Central regions of white matter tracts were more "mature" and organized than peripheral cortical regions, with lower MD and higher FA values. This pattern was also observed with T1 and T2 signal intensity, central regions of each white matter tract had higher T1 intensity and lower T2 intensity than peripheral cortical portions. Comparison of the splenium and the genu is consistent with the posterior to anterior maturation of white matter described in previous studies. In both the central and peripheral cortical regions, FA is higher, T1 signal intensity is higher, and T2 signal intensity is lower compared to the genu, reflecting more mature white matter tracts. In the white matter tracts of the genu and cortico-spinal tract, we detected the expected age related changes in MD (decreasing with increasing age), FA (increasing with increasing age), and T2 signal intensity (decreasing with increasing age). In the genu and cortico-spinal tract, there was no significant change in T1 signal intensity with age. Therefore, T2 signal intensity appears to be more sensitive to maturational events, even before myelination is evident. The relationship of fiber tract diffusion properties to myelination is

not straightforward. We observed high degrees of fractional anisotropy that increased with age in the unmyelinated central regions of the genu and splenium, in fact the FA of the unmyelinated central splenium was significantly higher than the myelinated central region of the cortico-spinal tract. T2 signal intensity was positively correlated with MD and negatively correlated with FA in each region, T1 signal intensity was not correlated with either MD or FA.
Discussion: Our study indicates that white matter tracts can be highly organized, even before myelination is evident on structural MRI. This study also demonstrates the utility of quantitative tractography of DTI images in the study of white matter development. Overall, our approach provides very consistent MD and FA values within specific regions of white matter tracts and is able to detect small regional differences as well as small age related changes in the neonatal period and relate them to T1 and T2 signal intensity.

26. Temperamental and Neuroendocrine Moderators of the Relation Between the Serotonin Polymorphism (5-HTTLPR) and Socioemotional Functioning in Childhood and Adolescence
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Sponsor: Travel Awardee, ADAA, 2006

Background: A serotonin transporter gene polymorphism (5-HTTLPR) interacts with stress exposure to predict risk for psychopathology. Studies also suggest that behavior is shaped by interactions between the 5-HTTLPR and the neuroendocrine system's response to stress. Questions remain, however, concerning the mechanisms through which stress and the 5-HTTLPR interact during development. The current study examines the links between (a) the 5-HTT polymorphism, (b) a neuroendocrine index of stress reactivity during childhood, and (c) early temperament (Behavioral Inhibition; BI) in shaping socioemotional functioning in early childhood. In addition, exploratory analyses extend this work to the presence of depressive and anxious symptoms in adolescence.
Methods: Children from a longitudinal cohort (N=174) were selected at 4-months based on affective and motor reactivity. The predictive variables for analysis were selected at various age points throughout the study: (a) Children are classified into two 5-HTT groups: Short allele (SA) for short allele pairs (s/s or s/l; N=85 to date) and Long allele (LA) for long allele pairs (l/l; N=40 to date). (b) At age 7, stress reactivity was assessed using the salivary cortisol response to a speech task (N=83). (c) Early temperament was assessed using a composite measure of laboratory observations of BI at four age points (14 months through 7 years). Measures of reticence and social play while participating in a laboratory play session with 4 same-age, same-gender peers at age 7 were used to asses socioemotional functioning. As adolescents, socioemotional and psychiatric functioning was assessed through semi-structured clinical interviews (K-SADS) and self- and parent-report measures. Noted in this abstract are preliminary findings for self-report of depression (Child Depression Inventory; CDI) and anxiety (Self-Report for Childhood Anxiety Related Disorders; SCARED).
Results: An initial MANOVA examined patterns of reticence and social play at 7, as a function of gender, HTT, BI, and stress response. A main effect for HTT found that SA children had increased reticence ($F(1,56)=4.26$, $p=0.04$). This pattern was most acute for SA children with a large cortisol stress response ($F(1,56)=4.76$, $p=0.03$). An emerging trend also indicates that males in the longitudinal cohort are driving this finding ($F(1,56)=3.31$, $p=0.07$). For social play at 7, the sole main effect found that a large cortisol stress response was linked to low levels of social play ($F(1,56)=4.26$, $p=0.04$). To extend these findings, exploratory analyses assessed depressive and anxious symptomatology in the adolescents participating to date in the longitudinal study. For the CDI, an increased cortisol response was linked to higher levels of depression, ($F(1,30)=8.52$, $p=0.01$). This pattern was most acute in children high in BI ($F(1,30)=5.79$, $p=0.02$) or with the HTT polymorphism ($F(1,30)=4.97$, $p=0.03$). For the SCARED, a large cortisol stress response was linked to increased anxiety in children with the HTT polymorphism ($F(1,24)=4.07$, $p=0.05$). In addition, reticence at 7 was linked to increased anxiety for children high in BI ($F(1,24)=4.50$, $p=0.04$) or with the HTT polymorphism ($F(1,24)=7.11$, $p=0.01$).
Discussion: The data from the on-going longitudinal study supports the proposition that individual differences in stress reactivity may interact with the serotonin polymorphism to shape developmental trajectories. This study is also among the first to suggest that early temperament, such as BI, may also play a moderating role. The data suggest that multiple factors come into play across time to elicit similar developmental outcomes. These mechanisms may work to sustain underlying behavioral biases over time, even as their expression shifts to reflect the child's current developmental stage.

27. Neurocognitive Outcome of Lamotrigine in Pediatric Bipolar Disorder
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Sponsor: Ghanshyam Pandey

Background: The antiglutamatergic mechanism of action of lamotrigine may result in improvement in neurocognitive functioning in pediatric bipolar disorder (PBD). This is the first prospective lamotrigine study to examine the comprehensive neurocognitive profile in PBD with an aim to primarily identify the effects of pharmacotherapy.
Methods: There were 65 subjects aged 13± 3 years including PBD patients (n=32) and healthy controls (HC) (n=33) matched on age, sex, race, socioeconomic status, IQ and reading ability. Patient population belonged to bipolar type I and II, with (hypo)manic or mixed episodes. All subjects completed tests on attention, executive function, attention, verbal learning, working memory and emotion recognition before and after 16 weeks of trial period. PBD patients received lamotrigine partial doses during the initial ramp up phase of 8 weeks and full and stable dose for the latter 8 weeks. Atypical antipsychotics were used as rescue measure only during the ramp up phase of lamotrigine. Data analysis included conversion of test scores to standardized z-scores relative to the baseline performance of the healthy comparison group to provide a standardized way to combine test score to form composite indices of neurocognitive domains. Prior to computing the z-scores, distributions of scores were normalized using log, square root, or linear transformations if needed. Scores for each neuropsychological domain were computed as the mean of the test scores comprising each neuropsychological function. Summary measures were calculated for executive function, attention, verbal learning, and working memory. The longitudinal course of clinical symptomatology was assessed using one-way repeated measures ANOVA for global YMRS and CDRS scores.
Results: Final mean dose of lamotrigine at the end point was 212.25 mg. On the clinical outcome measures, PBD patients significantly improved on the scores from baseline to the end point on young mania rating scale (Pre- test score: 21.74; Post- test score:5.35; $p<.001$) and on child depression rating scale (Pre- test score: 51.5; Post- test score:24.7; $p<.001$). Working memory deficits present at baseline were significantly improved by lamotrigine treatment relative to changes in healthy individuals. In the attention domain, although there were pretreatment deficits, there were no significant improvements after treatment. No deterioration was evident after treatment in any domain. Facial emotion recognition improved with treatment in patient group, especially for happy child faces relative to angry and adult facial emotions.
Discussion: Lamotrigine monotherapy resulted in no decline in neurocognitive functioning in PBD patients. There is significant improvement in working memory with lamotrigine therapy in PBD patients. There is distinct improvement in facial emotion recognition with

treatment. These results have direct clinical implications in treating PBD patients especially given the inherent cognitive deficits and treatment related cognitive dulling often seen in this patient population.

28. Effects of Early Experience and Lack of Social Support on the Endocrine and Behavioral Responses of Rhesus Macaques (Macaca mulatta) to Separation Stress

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Sponsor: Richard Meisch

Background: Research with both humans and animal models suggests that early adverse experience alters the development of hypothalamic-pituitary-adrenal (HPA) axis functioning and behavior, changes that have implications for the development of depression and anxiety disorders. Furthermore, social support in the form of affiliative contact with family members and/or peers has been shown to reduce the effects of stress. This study investigated the effects of early rearing history and level of social support on the endocrine and behavioral response to social separation stress in rhesus macaques.
Methods: Six-month old monkeys, reared either by their mothers in larger social groups (mother-reared), or in smaller groups limited to only same-aged peers (peer-reared), underwent four consecutive four-day long separations from their respective attachment sources (mothers or peers). Among the mother-reared subjects, infants with social support remained in their respective social groups while the mother was removed; Infants without social support were removed from the social group along with their mothers and housed individually in cages separate from their mothers. The HPA axis and behavioral responses to social separation were compared among the three groups of subjects (peer-reared, mother-reared with social support, and mother-reared without social support) using factor analysis to summarize the behavioral data, and ANOVA to compare ACTH, cortisol, and behavioral measures.
Results: Peer-reared monkeys showed lower basal ACTH ($p = 0.0002$) and cortisol ($p < 0.0001$) levels compared to both groups of mother-reared monkeys, but significantly higher cortisol responses to the acute phase (day 1) of social separation across the four weeks of separations (repeated measures ANOVA, $p < 0.0001$). Mother-reared monkeys without social support displayed higher ACTH responses to the acute phase of separation compared to mother-reared monkeys with social support (repeated measures ANOVA, $p < 0.0001$). However, mother-reared monkeys without social support showed severe blunting of ACTH levels during the chronic phase (days 2-4) of separation across all four weeks (repeated measures ANOVA, $p = 0.0132$) compared to both other groups. Factor analysis of the behavior data yielded three factors for each of the two separation phases (acute and chronic): despair, anxiety, and explore environment. Peer-reared monkeys showed the highest levels of despair during both the acute ($p < 0.0001$) and chronic phases ($p < 0.0001$). Mother-reared monkeys without social support showed the highest levels of anxiety during the acute ($p < 0.0001$) and chronic phases ($p < 0.0001$). Mother-reared monkeys with social support showed attenuated behavioral as well as HPA axis responses to separation stress.
Discussion: These findings indicate that early adverse experience may alter the development of the HPA-axis response system, which not only plays an important role in the stress response but is also implicated in the development of psychopathology. Lack of social support during times of stress may also alter the functioning of the HPA-axis, and therefore is also an important factor in the risk for psychopathology. This research was carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

29. Serotonergic Compensation Within the Rat Medial Prefrontal Cortex After Neonatal 6-OHDA Lesions

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Adolescence and early adulthood are critical periods in neurodevelopmental hypotheses of psychiatric disease. Maturation of the serotonergic (5HT) and dopaminergic (DA) systems appear to be dynamically active during these periods of neuropsychosocial development. In our laboratory, we have demonstrated that neonatal lesions of the serotonergic system result in an increase in dopaminergic fiber density within the adult medial prefrontal cortex (mPFC); whereas lesions of the dopaminergic system result in a decrease in serotonergic fiber density in this region. Of note however, is that the serotonergic innervation after 6-OHDA lesions, while reduced, is nevertheless substantial in adulthood. Here, we further describe a developmental phenomenon of "compensatory" serotonergic innervation occurring during adolescent and early adulthood periods of development.
Methods: On postnatal day 5 (P5), subjects received intracisternal injections of either vehicle only (10 mL saline ascorbic acid) or 10 mL 6-hydroxydopamine (6-OHDA) at a concentration of 50 mg/ml in saline with 10 mM ascorbic acid. On P40 and P70 animals were processed for serotonin fluorescence and peroxidase immunohistochemistry. A Bioquant OS/2 Image Analyses system was used to calculate the fiber density of 5HT-labeled processes within each cortical layer of the infralimbic and cingulate (Cg3) regions.
Results: P40 post-weanling animals that received neonatal 6-OHDA lesions at P5 were deficient in DA fibers and virtually devoid of 5HT fibers within the mPFC. At P70, however, a dramatic increase in 5HT fiber density was seen across all cortical layers of Cg3 and IL. This suggests mechanisms permitting vigorous plasticity of the serotonergic system, and perhaps others, during the late postnatal and early adulthood periods.
Discussion: While fiber density was diminished in P70 animals relative to sham-lesioned controls, P40 mPFC was virtually devoid of serotonergic fibers. Thus, in this lesion model, there appears to occur a dramatic increase in serotonergic sprouting within mPFC between P40 and P70. This developmental period also represents the interval during which behavioral normalization occurs after neonatal dopaminergic lesions (e.g. increased motoric activity). We propose a possible association between behavioral normalization and this neural plasticity. We also elaborate on our previously presented trophic model of monoaminergic developmental interaction.

30. Effects of Modafinil on Brain Regions Underlying Cognitive and Emotional Information Processing: An fMRI Study in Normal Healthy Volunteers

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Background: Decreased prefrontal cortical efficiency or signal-to noise of physiologic responses during working memory tasks for a fixed level of performance (as measured by the degree and extent of BOLD fMRI response) may underlie certain cognitive deficits such as those observed in patients with schizophrenia and Parkinson's disease, as well as in normal aging. Variations in monoamine levels, and in particular DA, have been implicated in the stabilization and focus of prefrontal cortical networks leading to enhanced cortical efficiency. Modafinil is a psychoactive drug marketed for the treatment

of excessive day-time sleepiness associated with narcolepsy. While its mechanism of action remains elusive, modafinil is thought to interact to some degree with adrenergic, GABAergic and dopaminergic neurotransmitter systems, possibly via a histaminergic mechanism, and to improve cognition without producing the anxiogenic effects of other monoaminergic stimulant drugs. In this study we assessed the ability of modafinil to improve efficiency in prefrontal cortical function and explore its effect on amygdala reactivity during emotional information processing, a measure thought to reflect anxiogenic liability.
Methods: We performed a double-blinded placebo controlled trial with modafinil (100 mg/day for 7 days) following a within-measures, counter-balanced study design. Twenty-six healthy normal volunteers (12 males, 14 females; Mean age = 35 years) underwent BOLD fMRI (3T) while performing a PFC-dependent task (the N-back working memory task) and while engaged in the perceptual processing of facial expressions with negative emotional valance.
Results: During the 2-back portion of the N-back working memory task, paired t-test using SPM2 revealed a significant main effect of modafinil on cortical efficiency. There was greater cortical activation in brain regions subserving working memory during the placebo condition relative to modafinil. Significant differences were found when comparing activation levels on placebo>modafinil in: 1) the anterior cingulate (BA 32, p=0.004, uncorrected), 2) the anterior PFC (BA 10, p= 0.007, uncorrected), 3) the dorsolateral PFC (BA 9, p=0.011, uncorrected), and 4) the ventrolateral PFC (BA 44,45,47, p=0.004, uncorrected). This was observed in the absence of a significant difference in accuracy or reaction time across the two drug conditions. In addition, both conditions produced significant activation of the amygdala during the processing of emotional faces, as would be expected. However, compared to the placebo condition, there was significantly less amygdala activity during the modafinil condition (right amygdala, p= 0.03, FDR-corr) without significantly impacting the Profile of Mood State (POMS) scores.
Discussion: These results suggest a unique effect profile of modafinil on information processing during these two tasks. Modafinil enhances cortical efficiency during working memory tasks, but, unlike other monoaminergic stimulants with similar effects on prefrontal cortical network systems, modafinil does not enhance (in fact it decreases) amygdala reactivity to emotional stimuli, suggesting no anxiogenic liability. Its ability to concomitantly enhance cortical focusing and reduce amygdala responses, at least at the fixed dose regimen used here, suggests that modafinil could have a number of useful neuropsychiatric applications, such as improving cognitive deficits related to cortical inefficiency and perhaps even alleviating symptoms associated with exaggerated amygdala responses.

31. fMRI Correlates of Formal Thought Disorder in Schizophrenia During Controlled versus Automatic Word Production

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Background: Although the link between formal thought disorder (FTD) and semantic memory is well established, only recently have functional brain correlates been examined. The current study used the Communication Disorders Index (CDI) to measure FTD, and functional magnetic resonance imaging (fMRI) to measure regional brain activity in 12 patients with schizophrenia during a cued overt word production paradigm.
Methods: Controlled processing was investigated by manipulating retrieval effort [automatic – (OS) versus effortful (SC)] and switching demand (switch versus no-switch). CDI scores were obtained from 10 minute speech transcripts by two raters with established reliability (ICC > .85). Resulting CDI frequencies (per 100 words) revealed mild to moderate levels of FTD (mean=1.8, SD=1.1, range 0.1-4.1) in these clinically stable medicated patients. Images were acquired on a 3 Tesla Siemens scanner using compressed image acquisition to allow for paced overt word production. Images were pre-processed in SPM-2, and a two-stage random effects analysis tested within and between group contrasts. There were no group performance differences. Relationships between FTD and fMRI were tested with a simple regression in SPM2.
Results: During automatic processing (OS) there were minimal correlations. However, during effortful retrieval (SC) higher levels of FTD were associated with reduced activation in bilateral anterior cingulate, right prefrontal and bilateral parahippocampal gyrus. Adding a switching demand produced an inverse relationship in which fMRI activation was greater in more thought disordered patients in bilateral prefrontal, left middle and inferior temporal, and left superior parietal regions.
Discussion: These results suggest a complex relationship between semantic memory function and FTD that is mediated by the nature of controlled processing demands.

32. Three-Dimensional Mapping of Hippocampal Anatomy in Unmedicated and Lithium-Treated Patients with Bipolar Disorder

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Background: Memory impairments are frequently reported in patients with bipolar illness, suggesting underlying hippocampal pathology. However, hippocampal volume deficits are inconsisently observed in bipolar disorder. Here we used surface-based anatomic mapping to detect features of hippocampal anatomy in bipolar patients treated with lithium relative to matched control subjects and unmedicated patients with bipolar disorder.
Methods: High-resolution brain magnetic resonance images were acquired from 33 patients with bipolar disorder (21 treated with lithium and 12 unmedicated), and 62 demographically matched healthy control subjects. Three-dimensional parametric mesh models of the hippocampus were created from manual tracings of the hippocampal formation.
Results: Total hippocampal volume was significantly larger in lithium-treated bipolar patients compared with healthy controls (by 10.3%; p=.001) and unmedicated bipolar patients (by 13.9%; p=.003). Statistical mapping results, confirmed by permutation testing, revealed that the right hippocampus was significantly atrophic in regions corresponding primarily to CA1 fields in unmedicated bipolar patients, compared to both normal controls (p=.01) and lithium-treated patients with bipolar disorder (p=.03).
Discussion: Atrophy in subregions of the hippocampus in unmedicated bipolar patients suggests a possible neural correlate for memory deficits observed in this illness. Moreover, our findings indicate that hippocampal volume is preserved in lithium–treated bipolar patients, which may reflect postulated neuroprotective effects of this agent.

33. Changes in Brain Metabolites Associated with Risperidone Treatment of Asperger's Disorder

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Sponsor: David S. Janowsky

Background: From a progression of our initial published studies of risperidone treatment of Asperger's Disorder, we have recently extended our work to include magnetic resonance spectroscopy characterizations of brain metabolites before and after treatment.
Methods: To date, we have examined 13 patients undergoing magnetic resonance spectroscopy prior to the implementation of a 12

week course of risperidone. Measures of prefrontal ratios of N-acetylaspartate (NAA), creatine (Cr), and choline (Cho) were obtained in both the left and right hemisphere by MRS.
Results: In these subjects, significant (F = 8.8, p = .025) improvements were observed with risperidone treatment in social functioning, measured by the modified Asperger's Syndrome Diagnostic Scale, replicating our previous findings. Examination of the brain metabolite data indicated a treatment effect on normalization of choline asymmetry. Before treatment, we observed higher choline ratios in right prefrontal cortex as compared to left, whereas after treatment, the choline concentrations were nearly identical on the right and left prefrontal cortex. This change was statistically significant on repeated measures ANOVA using SANS (Scale for the Assessment of Negative Symptoms) score changes as the covariant (F = 42.13, p<0.025). In addition, there was a trend for improvement in SANS scores to significantly interact with the normalization of choline ratios (F=12.05, p<0.075). Also, there was a negative correlation between the pretreatment choline concentrations on the left and the post-treatment choline concentrations on the right (r = .90, p = 0.10), and similarly, the pretreatment choline concentrations on the right and the post-treatment choline concentrations on the left (r = -.90, p < .025). Post-treatment choline concentrations on the right were significantly correlated with post-treatment SANS scores, controlling for differences in gender and initial SANS ratings (r = 0.83, p < .05), such that lower right choline concentrations were associated with lower negative symptom scores.
Discussion: These data are consistent with the hypothesis that risperidone may normalize choline asymmetries in prefrontal cortex in Asperger's patients, with high right brain choline concentrations being associated with greater negative symptoms, with changes in such associated with negative symptom improvement in Asperger's patients treated with risperidone.

34. Evidence of Regional Alterations in the Neurodevelopment of Psychostimulant-Naïve ADHD Children: A Multi-Voxel *In Vivo* ^{31}P Spectroscopy Study

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Sponsor: Chris Ellyn Johanson

Background: Attention-deficit/hyperactivity disorder (ADHD), is one of the most prevalent neurodevelopmental behavioral disorders. *In vivo* phosphorus (^{31}P) spectroscopy is a noninvasive technique that can directly assess the metabolism of membrane phospholipids (MPL) and high-energy phosphates in multiple brain regions, and is sensitive to neurodevelopment changes. Specifically, phosphomonoester (PME) levels (i.e., MPL precursor levels) reflect the mass of cellular MPL's or the proliferation content of dendrites and synaptic connections. In a recent study, we have shown deficits in MPL precursor levels in the basal ganglia (BG) and prefrontal (PF) regions of non-psychostimulant-naïve ADHD children compared to healthy children (HC)[1]. The purpose of this study is to assess regional differences in psychostimulant-naïve ADHD (*psn*-ADHD) children (that are relatively younger) as well as age effects compared to HC. We hypothesized greater MPL deficits in the relatively older psn-ADHD subjects compared to the younger children suggesting a lack of normal, progressive development in ADHD.
Methods: Thirty-one psychostimulant-naïve children with DSM-IV ADHD [25M+6F; mean age 8.1±1.2 yrs; 15 with the combined type and 16 with the predominantly inattentive type], and 36 HC (27M+9F; mean age 8.0±1.3 yrs) participated in this study. There were 7 *psn*-ADHD subjects who had a comorbid ODD and 2 subjects with both ODD and CD diagnoses. The freely mobile MPL precursor levels [*free*-PME] and 4 other metabolites [MPL breakdown products *free*-PDE, phosphocreatine, adenosine triphosphate, and inorganic orthophosphate] were quantified in the PF, BG, inferior parietal (IP) and 3 other regions using a multi-voxel, *in vivo* ^{31}P spectroscopy technique[1]. A generalized linear regression model (SAS Institute Inc., PROC GENMOD) with subject group, age and hemisphere as the main effect terms was used to test bilateral group differences in each region. A second model with an additional subject group-by-age interaction term was used.
Results: Bilateral regional effects: The *free*-PME levels were significantly lower in the BG (p=0.029) and significantly higher in the IP (p=0.038) of *psn*-ADHD subjects compared to HC. There were no other significant group differences in metabolite levels in the different regions. **Age effects:** There was a significant age-by-group interaction with *free*-PME in the PF (p=0.012) and IP (p=0.028) region as a result of greater *free*-PME contrasts between subject groups in the older *psn*-ADHD children.
Discussion: The MPL precursor level deficit in the BG across the *psn*-ADHD children suggests a reduction in mass of cellular MPL's due to a possible underdevelopment of neuronal processes and synapses in ADHD that is specific to the BG. Additionally, the MPL precursor levels are lower in the PF and higher in the IP compared to HC but only in the older *psn*-ADHD subjects. These age dependent effects suggest a deviation in the developmental trajectory possibly due to a lack of progressive development of neuronal processes and synapses in the PF and IP regions of ADHD children. The interpretation, however, is limited by cross-sectional observations and future longitudinal studies are warranted to truly assess progressive neurodevelopmental deviations in ADHD children. [1]Stanley et al., Psychiatry Research: Neuroimaging, 2006, (in press).

35. Acute High Dose D-Cycloserine Blunts Early Amygdala Response to Facial Expressions

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Sponsor: Roger K. Pitman

Background: D-cycloserine (DCS, an NMDA partial agonist) administered in the basolateral amygdala has facilitated extinction. In fMRI studies, amygdala activation habituates to repeated facial expressions, yielding significant differences between early and late periods of response. We examined DCS effects on amygdala habituation when processing repeated facial expressions and hypothesized that the DCS group would exhibit greater amygdala habituation, imitating facilitated extinction.
Methods: Fourteen healthy males (M=30.0, SD=8.7) were studied. In this double-blind study, individuals randomly received 500 mg of DCS or placebo approximately 1.5 hours prior to fMRI acquisition. All participants viewed four separate runs, consisting of a single 80-second block of facial expression (happy or fearful) bracketed by two 20-second fixation blocks. In each run, a single Ekman face was repeatedly presented. Thirty coronal slices were acquired on a Siemens Trio 3.0T scanner using a gradient echo T2*-weighted sequence (TR/TE/flip angle/slice thickness=2.0sec/30ms/90o/3mm). FMRI data were analyzed using the standard processing stream of the Martinos Center for Biomedical Imaging (Charlestown, MA). After preprocessing, functional data were modeled using a gamma function and averaged across participants according to condition (i.e. fearful early, fearful late, happy early, happy late). Using an anatomic region of interest (ROI)-based approach, the response in the left and right amygdala was examined separately. Percent signal change for each condition versus fixation was extracted for each participant and entered into a repeated measures ANOVA using emotion (fearful, happy) and period (early, late) as within subject factors and between-group factors (DCS, placebo).
Results: No group differences in post-scan subjective ratings of recognition, valence or arousal associated with the facial expressions

were reported (all p>0.35). Compared to the DCS group, the placebo group exhibited greater habituation in the left [period x group interaction: F(1,12)=11.42, p<0.005] and right amygdala [period x group interaction: F(1,12)=5.79, p<0.033]. Compared to the placebo group, the DCS group exhibited a blunted amygdala response in the early period [left: t(12)=1.92, p<0.079, right: t(12)=2.24, p<0.045], albeit the left amygdala effect was at trend level significance. No significant group differences were detected in the late period [p=0.38].
Discussion: Contrary to our original prediction, the placebo group exhibited greater amygdala habituation than the DCS group. Interestingly, healthy individuals exposed to an acute high DCS dose exhibited a blunted amygdala response. Given these findings, acute high doses of DCS (i.e. 500 mg) may act as an NMDA anatagonist. Another fMRI study using ketamine, an NMDA anatagonist, produced similar findings, a blunted amygdala response to facial expressions. Future studies should examine the dosage-dependent effects of DCS (i.e. 50, 100, and 500 mg) on amygdala activation to repeated facial expressions. Supported by NARSAD (CW)

36. Prefrontal Cortical Inputs to the Central Amygdaloid Nucleus in Primates
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Background: The prefrontal cortex (PFC) comprises 30% of the human brain and dictates social and emotional function and major cognitive skills, such as problem solving and decision-making. Because of its role in modulating social/emotional behavior, one connection that is particularly interesting is that with the amygdala. The amygdala plays a major role coding the salience of external stimuli, and projects to brainstem and hypothalamic sites involved in the expression of emotional behaviors. In this study we examined how the PFC influences the central amygdaloid nucleus, a key output site, in Macaques. We hypothesized that the PFC would be positioned to modulate this key output region.
Methods: We placed 7 injections of retrograde tracers into the central amygdaloid nucleus in adult monkeys using stereotaxic techniques. Brains were sectioned and tracers visualized using immunocytochemistry. Injection sites were then analyzed with respect to their position within the central nucleus and adjacent structures, taking into account placement within specific central nucleus subdivisions. The distribution of retrogradely labeled neurons in the prefrontal cortex was then charted at the microscope using a camera attachment and computerized mapping program (Neurolucida).
Results: The cortical areas that project most densely to the central nucleus as a whole are areas 25 (anterior cingulate), 14, and the agranular insula(Ia). With respect to specific subregions, the broadest inputs from the prefrontal cortex are to the medial subdivision of the central nucleus (CeM) and the interstitial nucleus of the posterior limb of the anterior commissure (IPAC), a transitional zone between the amygdala and striatum. These areas receive strong input from areas 25, 14, and Ia, a moderate input from areas 11, 12, 13, 24a/b, and a small input from areas 9 and 46. In contrast, the lateral core of the central nucleus (CeLcn) receives relatively restricted inputs, mainly from the agranular insula.
Discussion: The PFC has a direct projection to the central amygdaloid nucleus in primates based on retrograde studies. However, there is a differential input to specific subdivisions, reflecting the differences in output paths of these subregions. The CeM and the IPAC, which receive the broadest PFC innervation, form the main outputs to the brainstem and hypothalamus. Thus, the CeM and IPAC are positioned to have their outputs 'fine tuned' by broad regions of the orbital and medial PFC and insula. In contrast, the CeLcn plays an important 'gatekeeper' role in inhibiting the CeM and IPAC outputs, and has relatively few outputs outside the amygdala. Thus, the CeLcn, the internal 'gatekeeper' of central nucleus outputs, is mainly modulated by the agranular insula. Results will be discussed with respect to functional abnormalities of specific PFC regions in psychiatric disorders.

37. Discriminant Analysis of PIB PET Imaging Studies
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Background: Pittsburgh Compound-B (PIB) positron emission tomography (PET) imaging studies have demonstrated robust group differences in PIB retention between Alzheimer's disease (AD) and control subjects in areas of cortex that is consistent with the known regional amyloid deposition in AD. The present work extends previous analyses by applying the multivariate partial least squares (PLS) analysis to a large group of 46 subjects, that includes 21 controls, to evaluate its usefulness for the classification of "PIB-positive" and "PIB-negative" status.
Methods: Twelve AD subjects (69±9 yrs, MMSE-range: 18-28), 13 MCI (70±9 yrs, MMSE-range: 23-29), and 21 control (71±13 yrs includes 3 controls 39-45 yrs, MMSE-range: 26-30) subjects were studied with PET imaging (ECAT HR+, 10-15 mCi, 90 min). Magnetic resonance imaging was performed for region definition and spatial normalization (of individual data to an anatomical template). The PIB data were analyzed using the Logan graphical method with cerebellar (CER, reference region) data as input. Amyloid deposition was assessed via regional distribution volume (DV) ratio (DVR) measures of PIB retention. Regional values and voxel maps of the PIB DVR were generated. The PLS analysis applied a singular value decomposition to the X-Y cross-block covariance. The PLS analysis yielded a global overall brain score for each subject that was related to that individual's PIB DVR image values. A standard k-means cluster analysis was used to perform objective classification of subject groups.
Results: For the PLS analysis, the first singular component accounted for 96% of the covariance, while only 3% was accounted for by the second component. The first component PLS scores completely separated AD and control groups, with the exception of one control (PIB+) for whom primary cortical PIB DVR values exceeded the control mean by 1SD. A 2-cluster analysis yielded a "PIB-positive" (12 AD, 8 MCI subjects, PIB+ control) and a "PIB-negative" (20 control and 5 MCI subjects) group. A 3-cluster analysis yielded "PIB-positive" (10 AD, 3 MCI), "PIB-intermediate" (2 AD, 5 MCI, and PIB+ control), and "PIB-negative" (20 control and 5 MCI subjects) groups.
Discussion: The PLS results were in general agreement with previous regional analyses and indicated a variable range of PIB retention among MCI. Voxel-based PLS may prove useful for the classification of subjects on a spatial basis and help to better understand PIB retention for those subjects in the "PIB-intermediate" range, as well as those in the "PIB positive" and "PIB negative" groupings. This work was supported by NIA, MH070729, Dana Foundation, Alzheimer's Association, GE Health Care.

38. Learning to Trust: fMRI of Economic Exchanges in Individuals with Social Phobia
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Background: In order to avoid rejection, humans are likely to have developed strategies and neural circuits to assess the likelihood of positive/negative outcomes from social interactions. This ability is particularly salient when outcomes are unclear or inconsistent, making prediction more difficult. Such instances should then command

more vigilance and evoke neural circuits to detect salient stimuli of uncertain value. Individuals with generalized social phobia (GSP) exhibit anxiety and overestimate risk when the outcome of the social situation is unknown or ambiguous. Prior studies have employed static faces, but not dynamic social interactions, to probe the neuroanatomy of social anxiety.
Methods: We coupled event-related 3T fMRI and the 'trust' game to examine brain activation during a simulated social interaction. Thus far, twenty-four participants (8 GSP and 16 healthy controls [HC]: ages 21-49) have played a modified multi-round version of the trust game, in which subjects engage in economic decision making (whether to keep or invest money) with partners (3 simulated, anonymous [face obscured] human partners and 1 simulated computer partner), whose reactions inform the subjects about the probability of reciprocity, which is operationalized as the frequency that the invested dollars (now doubled) would be shared by the partner. Unbeknownst to the subjects, this frequency (over 20 rounds) was fixed at 75% (Fair Person), 50% (Unpredictable Person), 50% (Unpredictable Computer), and 25% (Unfair Person). Through trial and error, subjects learn about the reputation and identity of their partners (human and computer) over iterative exchanges. Using SPM5, we initially examined group differences in brain activity in the amygdala (previously implicated in the pathophysiology of GSP) during processing of partners in a random effects factorial model using a 2 (Group: GSP, HC) x 4 (Partner: Fair Person, Unfair Person, Unpredictable Person, Unpredictable Computer) ANOVA.
Results: The manipulation affected the subjects' decision to keep (or invest = transfer of money), such that there were significant differences in frequency between 'invest' and 'keep' decisions when subjects were playing with Fair (invest > keep) and Unfair (keep > invest) partners, but not with Unpredictable (human/computer) partners. The fMRI results revealed a significant Group x Partner interaction in bilateral amygdala (Left: [-16, 0, -26], $F[2,66]=4.57$; Right: [12, -6, -22], $F[2,66]=4.77$). Follow-up whole-brain and ROI-based t-tests showed that GSP subjects exhibited greater left ($t[22]=4.19$) and right ($t[22]=4.27$) amygdala activation than HC subjects to the Unpredictable Person, but no such difference was observed in response to either the Fair or Unfair Person. No difference in amygdala activation was detected between GSP and HC groups in response to the equally unpredictable Computer partner.
Discussion: These preliminary results demonstrate that the amygdala is sensitive to decisions made during dynamic social interactions when the outcome is uncertain. Exaggerated amygdala activation in GSP subjects may reflect a magnified alert for unpredictable outcomes, warning that choices based on the information available carry more unknown and/or unanticipated (potentially disadvantageous) consequences. Interestingly, GSP subjects do not activate the amygdala more so than controls during decisions related to general risk, as represented by nonsocial exchanges with the Unpredictable Computer. These data suggest that amygdala hyperactivity (or activation) may be a function of ambiguous outcomes of a social, interpersonal nature. Analyses of the effect of subject-specific decisions and partner-specific outcomes on brain activity await a larger sample. These findings may elucidate a novel mechanism for the neuropathophysiology of GSP.

39. A PET Study of Tiagabine Treatment Implicates Ventral Medial Prefrontal Cortex in Generalized Social Anxiety Disorder

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Background: Recent neuroimaging studies have suggested abnormalities within cortico-limbic circuitry in patients with generalized social anxiety disorder (gSAD) compared to healthy control subjects (HC). However all of these studies involved either a symptom provocation or a cognitive activation paradigm. Preliminary data suggest that tiagabine, a selective gamma aminobutyric acid (GABA) reuptake inhibitor, may have anxiolytic effects. The central objectives of the present fluorodeoxyglucose positron emission tomography (FDG-PET) study were three-fold: (1) compare resting state regional cerebral metabolic rate of glucose uptake (rCMRglu) between cohorts of gSAD and HC, (2) examine changes in resting state rCMRglu from pre- to post-treatment with tiagabine, and (3) determine cerebral metabolic predictors of tiagabine treatment response in gSAD.
Methods: Twelve right-handed patients with a primary diagnosis of gSAD (6 male, 6 female; ages 22-58, mean 32.1) and ten age- and gender-matched HC subjects were studied. All gSAD patients met illness severity enrollment criteria; Liebowitz Social Anxiety Scale (LSAS) ≥50 (mean gSAD LSAS score = 83.1 ±14.0). The LSAS served as the primary clinical measure of treatment response during an open, 6-week, dose-escalating trial of tiagabine. One resting state FDG-PET scan was acquired in the HC cohort and two resting state scans were acquired in the gSAD cohort (at pre- and post-treatment time-points). Imaging data were analyzed with SPM99 (http://www.fil.ion.ucl.ac.uk/spm). Voxel-wise tests were performed to identify between-group (gSAD vs. HC) differences in rCMRglu, treatment related changes in rCMRglu (gSAD post-treatment vs. pre-treatment), and to identify cerebral metabolic predictors of tiagabine treatment response.
Results: Between-group analyses of FDG-PET data demonstrated significantly reduced rCMRglu within the ventral medial prefrontal cortex (vmPFC) in the gSAD cohort compared to the HC cohort. The same vmPFC locus exhibited a significant increase in rCMRglu in the comparison of gSAD post-treatment vs. pre-treatment FDG-PET scans. Further, the magnitude of treatment response (% LSAS improvement) was inversely correlated with pre-treatment rCMRglu within this same vmPFC region.
Discussion: The present findings across three different analyses converge to suggest a possible role for the vmPFC in the pathophysiology and treatment of gSAD; increases in vmPFC metabolism associated with tiagabine treatment appeared to correct pathologically reduced metabolism in this region. Moreover, given the purported pharmacological profile of tiagabine, these findings suggest that its therapeutic effects in gSAD may be mediated by GABAergic modulation specifically within the vmPFC.

40. Effects of Divalproex on Brain Chemistry, Morphometry, and Function in Adolescents At Risk for Bipolar Disorder

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Background: Divalproex (DVPX) has been found efficacious in treating adolescents with and at high risk for bipolar disorder (BD), but little is known about the effects of mood stabilizers on the brain itself. We sought to examine the effects of divalproex on the structure, chemistry, and function of these areas in children at high-risk for BD
Methods: Twenty-four children with a parent with BD, who themselves had mood dysregulation but not full BD (putative prodromal BD), were treated with DVPX monotherapy for 12 weeks. A subset of subjects and 6 healthy controls were scanned with MRI on a GE 3T scanner (10 MRI, 10 MRS and 6 fMRI) at baseline and after 12 weeks. Single voxel (8 cc) 1H-MRS was performed in left and right dorsolateral prefrontal cortex (DLPFC). The fMRI task used was based on viewing negatively and neutrally valenced emotional stimuli from the International Affective Pictures System (IAPS). BrainImage was used for morphometric analyses of 3D coronal SPGR series.
Results: Preliminary results indicate changes in activation of prefrontal and amygdalar areas after DVPX treatment. Specifically, at baseline, controls had greater activation in right amygdala when viewing negative vs. neutral IAPS pictures. After treatment, prodromal subjects had greater right DLPFC activation compared to controls performing the same task. Left DLPFC NAA/Cr was no different

from healthy controls at baseline or follow-up. Right DLPFC NAA/Cr was not different from controls at baseline, but was significantly lower after treatment compared to controls (1.61 vs 1.69, $p < .02$). There were no significant differences in left or right DLPFC myo-inositol/Cr ratios. Finally, there were no significant changes in overall or prefrontal gray matter volume in prodromal subjects treated with DVPX.
Discussion: Adolescents with putative prodromal BD demonstrate abnormalities in prefrontal-limbic activation that change after treatment with DVPX monotherapy. Increases in prefrontal activation are consistent with theories regarding prefrontal control of mood, since all subjects in the fMRI study were clinical responders. Decreases in right DLPFC NAA/Cr were contrary to hypothesized increases and may reflect neuronal reorganization in that area. Absolute concentrations of NAA may provide further insight into effects of DVPX on prefrontal neurons. Lack of gray matter volume change after DVPX treatment may reflect our small sample size (type 2 error), need for greater length of treatment to show changes, or absence of effect of DVPX on this variable. Thus, while functional changes were commensurate wtih clinical improvement, despite abundant in vitro evidence of neuroprotective qualities of DVPX, we did not see evidence of neurogenesis in this small population of human subjects treated with DVPX. Longitudinal imaging studies in these high-risk populations are needed to understand effects of early intervention and prevention.

41. Test-Retest Reliability of Dorsolateral Prefrontal Cortical MRS Measurement of GABA and Glutamate/Glutamine at 3T
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Background: Evaluation of brain GABA and glutamate concentrations is of major interest in many neuropsychiatric conditions including stroke, epilepsy, substance abuse, depression, anxiety disorders, and schizophrenia. Recent studies using spectroscopic editing measurements of occipital lobe GABA have found significant deficits in depression (Sanacora et al., Arch Gen Psychiatry 1999) and in anxiety disorders. Postmortem studies have found pre- and postsynaptic alterations in schizophrenia in the dorsolateral prefrontal cortex (DLPFC) consistent with GABA transmission deficits (Volk et al., Cereb Cortex 2002). Recent work has shown high reliability of GABA and glutamate/glutamine ("Glx") measurements in DLPFC with a single-channel standard GE quadrature volume head coil and a fairly large acquisition volume (19.5 cc) (Kegeles et al., Proc Intl Soc Magn Reson Med 2006), as well as significant signal-to-noise (SNR) gains with a phased-array 8-channel coil in the same brain region (van der Veen and Shen, Proc Intl Soc Magn Reson Med 2005). The goal of the present study was to evaluate the potential for utilizing this improved SNR to reduce the acquisition volume by comparing test-retest reliability of the two configurations (single-channel with 19.5 cc voxel vs. 8-channel with 9 cc voxel) with the same acquisition time.
Methods: We studied 6 young adult healthy volunteers, 4 females and 2 males, to determine test-retest reliability of measurements of GABA and Glx concentrations in the DLPFC. All spectra were recorded in 26 minutes on a 3T GE 'EXCITE' MR system. Using internal landmarks on T1-weighted MRI scans, a voxel was reproducibly placed in the left DLPFC (middle frontal gyrus), angled parallel to the brain surface, with dimensions 1.0 cm x 2.0 cm x 4.5 cm in the 8-channel case. The J-edited spin echo difference technique (Rothman et al., Proc Natl Acad Sci USA 1993; Sailasuta et al., Proc Intl Soc Magn Reson Med 2001) followed by a frequency-domain nonlinear least-squares spectral fitting procedure were used to determine GABA and Glx in this DLPFC voxel, which were normalized both to the creatine-containing compounds (Cr) and to the internal water signal recorded simultaneously. Test-retest reliability of the measurements was assessed with the Pearson correlation coefficient (R), the percent coefficient of variation (%CV), and the intraclass correlation coefficient (ICC).
Results: Test-retest reliability using this methodology was found to be very high. For the 8-channel acquisitions, the Pearson correlation coefficient of test vs. retest for GABA normalized to internal water was $R = 0.993$ ($R^2 = 0.986$, $p < 0.0001$). Percent coefficient of variation was %CV = 5.2%, and ICC was 0.84. Similar reliability was found for Glx to internal water ratios, as well as for both GABA and Glx ratios to Cr. Test-retest reliability was comparable to the single-channel configuration with larger voxel size.
Discussion: These data show that DLPFC GABA and Glx can be measured with high reliability under the conditions of our study. Use of the improved SNR of the 8-channel system to reduce voxel volume allows a more purely gray matter volume to be sampled without degradation of signal reliability. These data suggest that reliable quantification of these critical neurotransmitters can be achieved in DLPFC with these methods in clinical studies. Acknowledgments: We are indebted to J.W. van der Veen, Ph.D. (NIH), R. Hurd, Ph.D. (GE) and S. Kohler, Ph.D. (GE) for assistance in porting the editing sequence from the GE 'LX' to the 'EXCITE' platform. Funding: Weill Cornell Medical College New Faculty Development Funds (DCS), NIMH K08 MH01594 (LSK), and Lieber Center for Schizophrenia Research.

42. Reduced Serotonin Synthesis Capacity in Male Subjects with Enduring Traits of Aggressive Behaviour Since Childhood
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Sponsor: Theodore Sourkes

Background: Low serotonin neurotransmission is one of several mechanisms central to the neurobiology of impulsive aggression. Recently, this group reported a marked reduction in serotonin synthesis capacity in corticostriatal pathways believed to regulate the expression of behavioral inhibition / disinhibition, in medication-free Character Disorder patients endowed with high impulsivity (Leyton et al., 2001). One limitation in interpreting the significance of this observation was that the use of a cross-sectional design, focusing on adult patients, whose complex clinical history is often reconstructed a posteriori and lacks a developmental perspective, can hardly resolve trait- from state-related abnormalities. The present study entailed a significant paradigm shift, from patient to population-based cohort studies of stable enduring traits since childhood. We specifically tested the hypothesis that adults, characterized by a childhood onset of physical aggression, have reduced serotonin synthesis capacity. Brain regional serotonin synthesis was indexed by Positron Emission Tomography (PET) and α-[(11)C]methyl-L-tryptophan blood-to-brain clearance / trapping (K^*).
Methods: Eight subjects (n=8) with and thirteen (n=13) subjects without childhood onset aggression were tested (mean age ± SD: 27.0 ± 0.7). All subjects were members of the Montreal cohort of kindergarten schoolboys (N=1037), followed at regular intervals since childhood for various developmental, behavioral, biological and psychosocial risk factors, and representative of four developmental trajectories for physical aggression (2 high physical aggression, 2 low physical aggression) (Nagin & Tremblay, 1999). Sixty-minute dynamic PET scans were conducted with an ECAT HR+, following intravenous administration of α-[(11)C]methyl-L-tryptophan (10mCi). Several pencil-and-paper behavioral measures (Buss-Durkey Hostility Inventory, Bar-On Emotional Quotient Inventory, Brown-Goodwin assessment for lifetime history of aggression) and tests of executive function were recorded. All subjects were genotyped for 5HTT, TPH2 and 5HT1A polymorphism. Impulsivity was indexed by the number of commission errors on a Go-No-Go task, a laboratory measure of behavioral inhibition.

Results: Subjective measures of aggression confirmed that individuals in the high physical aggression trajectory exhibited more aggressive behaviors during childhood ($F(1,19) = 5.50$; $p = 0.03$), adolescence ($F(1,19) = 9.93$; $p = 0.005$) but not in adulthood ($F(1,19) = 1.83$; $p = 0.19$), as measured by the Brown-Goodwin assessment for lifetime history of aggression. They also exhibited higher scores on the Buss-Durkee Hostility Inventory subscales physical aggression ($F(1,17) = 4.62$; $p = 0.046$) and hostility ($F(1, 17) = 5.53$; $p = 0.03$). There were no differences in the number of commission errors on the Go-No-Go task between trajectories. Remarkably, males with impulsive aggression since childhood demonstrated a marked bilateral decrease in normalized α-[(11)C]methyl-L-tryptophan trapping in the lateral orbito-frontal cortex ($p \leq 0.005$), as compared to the more favorable outcome trajectories. This was the only group difference in tracer trapping.
Discussion: Low serotonin synthesis capacity in the orbito-frontal cortex, may underscore in part, the developmental trajectories identified in the boys at risk for delinquency.

43. Plasma Fatty Acids and Suicide Attempts Associated with Regional Brain Glucose Uptake

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Sponsor: Maria A. Oquendo

Background: Polyunsaturated essential fatty acids are dynamic constituents of neuronal cell membranes. Fatty acid deficiencies and serotonin dysfunction have been implicated in mood disorders and suicide risk. Fatty acid deficiencies predict risk of suicide attempts in major depression but the mechanism is uncertain. Objectives: to 1) map associations between regional cerebral glucose metabolism and plasma phospholipid levels of docosahexaenoate and arachidonate in depressed subjects, 2) compare these associations with regional cerebral glucose metabolism differences between depressed suicide attempters and nonattempters, and 3) examine the effects of fenfluramine-stimulated serotonin release on these paradigms.
Methods: Design: [F-18]-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) three hours after placebo and fenfluramine administrations. Sample: Two overlapping groups of subjects in a major depressive episode, medication-free for 2 weeks: 1) subjects (N=29) who had plasma fatty acid measurements and FDG-PET; and 2) subjects (N=37) who had FDG-PET and follow-up for 2 years to record suicide attempts. Outcome Measures: Statistical parametric maps of regional cerebral glucose metabolism: 1) correlations with plasma phospholipid fatty acid levels, and 2) comparisons of subsequent suicide attempters with nonattempters.
Results: Regional cerebral glucose metabolism correlated positively with plasma phospholipid levels of docosahexaenoate and arachidonate in right temporoparietal cortex, and with arachidonate in cerebellum. Regional cerebral glucose metabolism correlated negatively with plasma phospholipid docosahexaenoate in left anterior cingulate, inferior and middle frontal cortex. Fenfluramine had fatty acid- and region-specific effects on regional cerebral glucose metabolism. Subjects (N=8) who made subsequent suicide attempts, compared with non-attempters, exhibited altered cerebral glucose metabolism in brain regions that overlapped fatty acid-correlated regions by 22-49%.
Discussion: This is the first PET study examining plasma fatty acid levels and human brain functioning in major depression. Relationships between plasma phospholipid fatty acids, serotonin, and brain activity could explain how dietary fatty acids may contribute to suicide risk by affecting regional brain functioning.

44. Using Motor and Prefrontal Cortex Interleaved Transcranial Magnetic Stimulation (TMS) BOLD fMRI to Compare the Mechanism of Action of Lamotrigine to Valproic Acid in Normal Volunteers

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Background: In earlier work we found that a single 325 mg oral dose of lamotrigine (LTG) raised the TMS motor threshold by 15%. LTG also diminished the local and distributed effects of motor cortex TMS as measured by interleaved TMS/BOLD fMRI. This study tests the prior results for replication and examines whether the LTG effects are specific to sodium channel blocking drugs. That is, does an equivalent dose of valproic acid (VPA), which does not inhibit voltage-gated sodium channels at therapeutic concentrations, show a similar effect to lamotrigine in the TMS/BOLD fMRI model? This is important since LTG and VPA have different efficacy profiles in the treatment of bipolar disorder, thus the model might provide new insight into the mechanism of action of the drugs in psychitric illness. . Thus, this initial protocol in healthy volunteers is designed to further develop a method that might then be applied to understand and differentiate the effects of LTG and VPA in controlling mood in bipolar patients. We hypothesize that in volunteers, TMS over both motor and prefrontal cortex will show a local and secondary increase (orbitofrontal cortex, hippocampus and anterior cingulate) in relative blood flow as measured by the BOLD signal following TMS. We hypothesize that immediately following a single oral dose of LTG; these healthy volunteers will have a reduction in the BOLD signal (compared to the sham day) in motor cortex, while having an increase in the BOLD signal in the limbic system. We also hypothesize that immediately following a single oral dose of VPA, the healthy volunteers will have neither an elevated resting motor threshold (RMT) nor a reduction in the BOLD signal in motor cortex. Furthermore, they will not have an increase in the BOLD signal in the limbic system during TMS over prefrontal cortex. We also investigate how the two drugs affect other cortical excitability measurements (cortical silent period-CSP and input/output curve) in healthy men.
Methods: 30 healthy young men (26 years average age) were enrolled and given, in a randomized and blinded fashion on three separate days (separated by a week), either a single dose of LTG 325 mg, or a single dose of VPA 1250 mg, or placebo. On each testing day, the RMT, CSP and input/output curve were assessed using TMS with Spike 2. This was performed at baseline and then 3 hours after administration of medication. Serum LTG and VPA levels were determined. Subjects were then scanned in a 3.0 T scanner with sense-head coil and intermittent TMS applied over the left motor cortex and in a separate run, over the left prefrontal cortex.
Results: Data acquisition is complete with data being analyzed. Full results will be presented at the meeting.
Discussion: The interleaved TMS/fMRI technique is a useful new method for understanding the regional brain effects of CNS active compounds, particularly those with inhibitory mechanisms.

45. Selectivity of the PET Radiotracer [^{11}C] NNC 112 for Dopamine D1 Receptors vs Serotonin 5HT2A Receptors: An Occupancy Study with Risperidone in Healthy Human Volunteers

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Background: There are 2 widely used PET tracers for imaging dopamine D1 receptors: [^{11}C] NNC 112 and [^{11}C] SCH 23390. While it has been known for decades that both have some affinity for serotonin 5HT2A receptors, the D1:5HT2A selectivity ratio reported in the *in vitro* literature was 100 to 200 fold, so that 5HT2A binding was

assumed to comprise a negligible fraction of the tracer signal. However, this property of the radiotracers remained untested in the more relevant setting of human imaging. Recently, we have observed in nonhuman primates that *in vivo*, the selectivity ratio is closer to 10 fold and that 5HT2A binding comprises 1/4 of the specific binding signal in cortex for both ligands. The present study was designed to verify this finding in humans by measuring the effect of acute doses of the antipsychotic drug risperidone, which has high affinity for 5HT2A and negligibly low affinity for D1, on [^{11}C] NNC 112 binding in cortex.
Methods: 7 healthy human volunteers (5 M, 2 F, age 28 ± 6 yr) underwent dynamic PET scans with [^{11}C] NNC 112 at baseline and again 2 hours after 2 mg of oral risperidone, a dose that putatively would block the majority of 5HT2A receptors. The specific to nonspecific equilibrium partition coefficient (V3", proportional to B_{max}/K_D) was measured in cortex and striatum under both conditions. V3" was derived by kinetic modeling of the dynamic data using the ligand concentration in arterial plasma as an input function. ΔV3", defined as 100%*(V3"(post risp.)/V3"(base)-1), was taken as a measure of the decrease in specific binding following the challenge.
Results: V3" decrease across cortical regions (anterior cingulate, dorsolateral prefrontal, medial prefrontal, orbitofrontal, temporal, parietal and occipital cortices) was 30 ± 4 % and was statistically significant in each region (paired t test), with significance surviving multiple comparisons correction. In striatal subregions (pre and post commissural caudate and putamen and ventral striatum) ΔV3" was not significantly different from 0 (3 ± 5% increase).
Discussion: Given the approximate 2:1 ratio of 5HT2A to D1 density in human cortex, the 30% decrease observed here is consistent with the 10 fold selectivity we previously observed in nonhuman primates. The lack of change in striatum, a region with high D1 and negligible 5HT2A, served as an internal control to demonstrate that the observed change in cortex was not due to changes in D1 binding. This study suggests that caution must be used in the interpretation of studies using [^{11}C] NNC 112 to measure cortical D1 tone in patient populations, as apparent D1 effects may be confounded by 5HT2A involvement. It also highlights the fact that effecitive affinities and selectivities of radioligands *in vivo* can be quite different than the values suggested by *in vitro* data. It remains for future work to see if the effect translates to humans with SCH 23390 as well.

46. Disruptions in Connectivity Between Frontal and Temporal Lobes in Schizophrenia: A Diffusion Fiber Tractography Study

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Background: Disruptions in white matter brain connectivity between the frontal and temporal cortices are thought to underlie at least some of the symptoms observed in schizophrenia. One of the major paths connecting the frontal and temporal lobes leads through the temporal stem. According to new imaging data, this anatomical structure, in addition to uncinate fasciculus (UF) also contains the inferior occipito-frontal fasciculus (IOFF), a long bundle connecting posterior temporal, parietal and occipital brain regions with the frontal lobes. The UF has previously been observed to be abnormal in schizophrenia, however the IOFF has not been investigated separately in schizophrenia.
Methods: To separate and investigate both UF and IOFF connections in schizophrenia, we used DTI (Diffusion Tensor Imaging), an in vivo method that uses an MR signal to probe the relative magnitude of the direction of water diffusion, thus detecting microscopic variations of white matter. In addition, we used fiber tractography, a method using the principal diffusion direction to compute the pathways of nerve fiber tracts. DTI scans were acquired from 61 participants, 27 patients diagnosed with schizophrenia and 34 controls. A fiber-tracing method was then used to estimate fractional anisotropy (FA) along the UF and IOFF fiber tract.
Results: Results of a repeated measures ANOVA revealed a tract by group, and tract by side interactions ($P(1,59) = 0.014$; $P(1,59) = 0.015$). Post-hoc t-tests revealed that mean FA in schizophrenics was significantly lower than normal controls in both the left ($P(1,59) = 0.019$), and right ($P(1,59) = 0.031$) IOFF, but not in left ($P(1,59) = 0.900$) or right ($P(1,59) = 0.612$) UF. In addition, both fiber tracts demonstrated negative correlations with negative symptoms (i.e., lower fiber integrity, more negative symptoms). Finally, cognitive measures differentiated IOFF and UF function in schizophrenia. More specifically, lower UF integrity was correlated with poorer memory performance, while lower IOFF integrity was associated with worse visual and spatial attention scores.
Discussion: These results provide quantitative evidence for a reduction of frontal-temporal connectivity in schizophrenia. They also point to IOFF as a possible source of this disturbance in connectivity, and confirm a relationship between structural and functional abnormalities observed in schizophrenia.

47. Neural Substrates for Processing Task-Irrelevant Sad Distracters in Adolescents

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Background: Neural systems related to cognitive and emotional processing were examined in adolescents using event-related functional magnetic resonance imaging (fMRI).
Methods: Ten healthy adolescents were scanned during an emotional oddball task, where subjects must detect infrequent circles (targets) within a continual stream of phase-scrambled images (standards). Sad and neutral images were intermittently presented as task-irrelevant distracters.
Results: As in adults, there was increased activation in the dorsal attention-executive system, including anterior middle frontal gyrus (aMFG), dorsal anterior cingulate (ACG), posterior cingulate (PCG), insula, and supramarginal gyrus (SMG), in response to task-relevant targets. Unlike adults, the adolescents exhibited stronger activation to sad images in the ventromedial prefrontal cortex (VmPFC), posterior middle frontal gyrus (pMFG), and several regions of parietal cortex. Across subjects, increased VmPFC activation to emotional distracters was associated with reduced activation in aMFG during target detection, suggesting that emotional information hampered cognitive processing in adolescents. Greater activation in the pMFG and PCG evoked by sad distractors was associated with greater activation in the aMFG during target detection, implying a possible inhibitory role for these regions.
Discussion: The extensive network activated while performing a cognitive task during the presence of emotional distracters suggests immaturity of cognitive over emotional processes during adolescence.

48. Associations of Brain White Matter Microstructure and Volumetric Changes with Executive Functions in Healthy Adolescents

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Sponsor: Kelvin O. Lim

Background: Animal, autopsy, and brain imaging studies converge to suggest that the brain continues to mature, albeit in subtle ways, throughout childhood and into adolescence. Adolescence is characterized by an increment in white matter volume and a gray matter volume decline (Sowell et al, 2001). These changes are particularly

notable in the frontal lobe relative to other cortical regions. In addition to volumetric changes, white matter microstructure becomes more refined between childhood and adulthood, perhaps due to increased myelination (Smithhorst, 2000). These changes are thought to stabilize neural circuits that support high-level cognition, including working memory, planning, and inhibitory control. These behavioral functions show a protracted developmental course throughout adolescence, as demonstrated by our laboratory (Conklin et al., 2006; Luciana et al., 2005; Hooper et al., 2004). Few studies have attempted to associate developmental changes in brain structure with concomitant cognitive advances. These associations are important to quantify, because various forms of psychopathology have been linked to neurobehavioral deficits as well as relative differences in brain structure and activity patterns. The current study presents brain imaging and behavioral findings from healthy adolescents, who are the focus of a longitudinal investigation.
Methods: Healthy adolescents, ages 9-21, completed a structural MRI scan using diffusion tensor imaging (DTI) as well as a neurocognitive task battery. All participants were screened to rule out psychopathology and neurological impairment. The task battery focused on behaviors mediated by dorsolateral versus ventromedial regions of the prefrontal cortex as well as the anterior cingulum. Indices of behavioral performance that reflect planning skills, response control, and working memory were quantified. From the brain scans, measures of gray matter volume, white matter volume, fractional anisotropy and trace diffusion were quantified throughout the brain and in dorsal versus ventral regions of the prefrontal cortex using region-of-interest and voxel-based methods.
Results: Within the prefrontal cortex, we observe age-related increases in gray matter volume in dorsal and ventral subregions as well as increases in fractional anisotropy. There are also age-related improvements in planning, inhibitory control and working memory. The age at which functional maturation is achieved varies by task. Notably, when these findings are associated, it is apparent that changes in brain microstructure and volume contribute to adolescent cognitive development above and beyond the influence of age alone.
Discussion: Findings will be discussed in terms of prevailing theories of brain development, which posit that the frontal lobe is one of the last regions to reach full functional maturity. Implications of these patterns for understanding psychopathological conditions that emerge during adolescence will also be discussed.

49. Classification of Schizophrenia and Bipolar Disorder Using Temporally Coherent Functional Networks

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Schizophrenia and bipolar disorder are currently diagnosed on the basis of a constellation of psychiatric symptoms and longitudinal course. The determination of a reliable biologically-based diagnostic indicator of these diseases (a biomarker) could provide the groundwork for developing more rigorous tools for differential diagnosis and treatment assignment. Recently, methods have been used to identify distinct sets of brain regions or "spatial modes" exhibiting temporally coherent brain activity.
Methods: Using functional magnetic resonance imaging data and a multivariate analysis method, independent component analysis, we combined two of these modes, the temporal lobe mode and the default mode, to discriminate subjects with bipolar disorder, chronic schizophrenia, and healthy controls.
Results: Temporal lobe and default mode networks were reliably identified from all participants. Classification results on an independent set of individuals reveal an average sensitivity and specificity of 90% and 95%, respectively.
Discussion: The use of coherent brain networks such as the temporal lobe and default mode networks may provide a more reliable measure of disease state than task-correlated fMRI activity. A combination of two hemodynamic brain networks show promise as a hemodynamic biomarker for schizophrenia and bipolar disorder.

50. The Effects of DAT, SERT and NET Gene Knockout on Behavior in Animal Models of Depression

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Background: Antidepressant drugs display varying potencies in blocking the plasma membrane transporters for dopamine (DAT), serotonin (SERT), and norepinephrine (NET), indicating potential roles for all three of these neurotransmitter systems in depression. Studies of the effects of DAT, SERT, and NET gene knockouts (KO) in behavioral models of depression may indicate the possibility that human gene variants that produce similar variations in monoamine transporter expression might also affect depressive phenotypes. Such effects might be expected because these manipulations chronically elevate extracellular monoamine levels. Indeed, previous studies have shown that some of these gene knockouts exhibit profiles in animal models of depression indicative of "antidepressant-like" effects. However, to facilitate relative comparisons of the contributions of each of these transporters to these phenotypes, we here report simultaneous comparisons in two animal models of depression: the forced swim test and the tail suspension test.
Methods: The forced swim test paradigm used here examined multiple behavioral endpoints in an attempt to separate effects on general activity from those specific to antidepressant effects, an important consideration in DAT KO mice in particular. In addition to immobility, swimming and struggling behavior were also measured. Mice were place in a 3 L cylindrical beaker (19 cm diameter, 14 cm water depth) for 15 minutes and then removed. On the following day they were placed in the beaker for 5 minutes and the test digitally recorded for scoring by a trained observer. In the tail suspension test, mice were suspended by the tail from a horizontal metal rod (8 mm diameter) for 3 minutes. The duration of immobility was measured by an observer with a stopwatch.
Results: As was previously reported, NET KO and especially DAT KO mice were found to have reduced immobility time in the FST, while SERT KO was without effect. Furthermore, in DAT KO mice increased immobility was accompanied by reduced swimming but profoundly increased struggling behavior. This was taken to indicate primarily an "antidepressant-like" effect, which is supported by the fact that DAT +/- mice, which are not hyperactive, exhibited a similar but less pronounced behavioral change. The effect of NET KO on immobility, while statistically significant, was much less pronounced than that observed in DAT KO mice, and the effect of NET KO on struggling was not significant, although there was a similar trend. In the tail suspension test a similar overall pattern of effects was observed in these knockout mice, although, in agreement with previous reports SERT reduced immobility in this test. However, as in the FST the effects of DAT were much more pronounced than the effects of NET, or in this case SERT as well.
Discussion: These "antidepressant-like" effects in DAT KO mice are not easily explained by confounding effects of other behavioral changes, such as the hyperactivity of DAT KO mice. These mice perform normally in a number of other tasks and the "antidepressant-like" profile was observed in both hyperactive homozygous DAT KO mice and in heterozygous DAT KO mice which are not hyperactive. Overall, these data support a greater role for DAT in baseline depressive phenotypes than has previously been appreciated. Interestingly, selective DAT blockers appear to have similar consequences in these models. Together, these data suggest that the investigation of human

gene variants that alter DAT expression might be warranted. (Support: NIDA-IRP/NIH/DHHS)

51. Neural Circuitry of Submissive Behavior and Treatment Response in Social Anxiety Disorder: Preliminary Findings

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Background: Fear of eye gaze is a common symptom of social anxiety disorder (SAD) and appears related to evolutionarily-conserved submissive behaviors, such as avoidance of direct gaze and vigilance for social threat. Threatening facial expressions activate neural circuits in SAD involved with perceiving social visual cues, but eye gaze stimuli have been little studied. This study assessed neural circuitry activation in SAD, before and after treatment, using fMRI with a novel set of face photo stimuli that realistically simulate eye motion into direct or indirect gaze. We hypothesized that in SAD patients, processing of faces with direct eye gaze would preferentially activate fear circuitry structures such as the amygdala and insula, associated frontal regions (rostral anterior cingulate and medial prefrontal cortex), and core areas of visual face processing (fusiform gyrus), and that after treatment with serotonin reuptake inhibitor (SSRI) medication, these regional activations would normalize.
Methods: Five patients with generalized SAD and 5 healthy control (HC) subjects, age 18-55, unmedicated and with no significant comorbidity, were presented blocks of visual stimuli of faces with gaze shifting toward subject (direct) or away (indirect), and they identified direction of gaze by button pressing. fMRI imaging was conducted with a 1.5T scanner, and gaze avoidance was assessed by continuously recording subjects' eye positions with an eye tracking device and analyzing % of fixations on the eye region of each stimulus. Three SAD subjects also completed a second scan after 12 weeks of paroxetine treatment, and 3 HC subjects completed a second scan after a 12 week interval.
Results: Results of subjects' first scans, analyzed with SPM2 for activation in response to direct > indirect gaze stimuli, demonstrate areas of significantly increased activation in the SAD group compared to HC subjects in amygdala, fusiform gyrus, insula, anterior cingulate and prefrontal cortex ($p<.01$). There was a trend for the mean difference in percentage of fixations on the eye regions (for indirect - direct gaze stimuli) to be higher for SAD patients compared to HC subjects (10.2+/-5.3 vs. 4.6+/-3.9; $t=1.9$, $df=8$, $p=0.09$). This suggests that SAD patients, compared to HC subjects, may have avoided direct gaze more than indirect gaze. SAD patients had a nonsignificantly lower percentage of fixations on the eye region in response to both indirect and direct gaze stimuli.
Discussion: These preliminary data demonstrate feasibility and support hypotheses of regional activations in the SAD group in response to direct vs. indirect gaze. If threat neurocircuitry activation is associated with direct eye gaze in SAD and decreased activation in these regions is associated with treatment response, this would support the response to dynamic direct gaze stimuli as a biomarker suitable for further study in animal models and humans with SAD and other disorders.

52. A Double Blind Comparison of Ziprasidone versus Sertraline and Haloperidol Combination Therapy for the Treatment of Major Depression with Psychotic Features

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Sponsor: K. Ranga Krishnan

Background: Although atypical antipsychotic medications have become first line treatment for psychotic symptoms, there is emerging evidence for antidepressant efficacy in mood disorders as well. Psychotic depression represents a severe major depressive syndrome typically treated with either an antidepressant/antipsychotic medication combination or electroconvulsive therapy. The purpose of the current study was to assess the efficacy and safety of subjects with psychotic depression treated with either ziprasidone or a combination of sertraline and haloperidol.
Methods: Subjects meeting DSM-IV criteria for major depressive episode with psychotic features were randomized in a 1:1 ratio to either ziprasidone monotherapy or a combination of sertraline and haloperidol and studied over a 12 week period. Rating scales included the 21-item Hamilton Depression Rating Scale, Brief Psychiatric Rating Scale and Clinical Global Impression Scales administered at screening, baseline, day 3 and after 1, 2, 3, 4, 6, 8, and 12 weeks of treatment.
Results: Seventy-two subjects were recruited at one of four sites (John Umstead Hospital/Duke University, Durham, N.C.; University of Southern California, Los Angeles, California; National Institute of Mental Health and Neurological Science, Bangalore, India; University of Alexandria, Alexandria, Egypt) and randomized to either a combination of sertraline and haloperidol (n=37) or ziprasidone monotherapy (n=35). Similar robust improvements in depressive and psychotic symptoms were observed under both treatment conditions. Mean BPRS scores in the ziprasidone and combination sertraline/haloperidol groups were repectively 51.0 ± 8.8 and 52.2 ± 9.6 at baseline and 29.1 ± 10.7 and 27.7 ± 13.4 at LOCF-endpoint. Mean HDRS-21 scores in the ziprasidone and combination sertraline/haloperidol groups were repectively 32.6 ± 5.8 and 31.9 ± 5.5 at baseline and 13.7 ± 8.3 and 11.0 ± 7.7 at LOCF-endpoint. Further data will be presented.
Discussion: Ziprasidone monotherapy resulted in similar robust decreases in psychotic and depressive symptoms as a combination of sertraline and haloperidol treatment in this study of psychotic depression. This study provides further support for the utility of atypical antipsychotics in the treatment of severe mood disorders. Further studies are warranted.

53. Psychosocial Functioning in Patients with Difficult-to-Treat Unipolar, Nonpsychotic, Major Depression

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Sponsor: Martin B. Keller

Background: While reducing depressive symptoms remains the primary goal when treating patients with major depression, psychosocial functioning is increasingly seen as an important outcome in gauging a patient's progress. This 2-site study examined whether risperidone augmentation for patients with difficult-to-treat major depression had an effect on psychosocial functioning and quality-of-life.
Methods: 97 patients who met criteria for unipolar, nonpsychotic major depression, and failed to respond or only partially responded to at least 5-weeks of open label antidepressant monotherapy were continued on their antidepressant medication and randomized to receive adjunctive risperidone (n=67) or placebo (n=33) for an additional 4-week treatment trial. Psychosocial outcome measures administered at baseline, and after two and four weeks of study medication included the Social Adjustment Scale (SAS), the Range of Impaired Functioning (LIFE-RIFT) scale, and the total and items scores of the Quality-of-Life (Q-LES-Q). Repeated measures analysis of covariance, logistic regression, and slopes analysis were used to examine rates of change in functioning between the two treatment groups, the treatment effect on change in functioning at each visit, the response to medication, and correlations of changes in depression with changes in psychosocial functioning.

Results: There was no consistent pattern of improvement of psychosocial variables over time. Risperidone augmentation, however, had a greater effect than placebo in many areas of functioning at some points in time. Compared to patients in the placebo group, patients in the risperidone group showed significantly greater improvement in SAS-social/leisure, SAS-family, LIFE-total, LIFE-satisfaction, and Q-LES-Q total, Q-LES-Q medication satisfaction, and Q-LES-Q overall satisfaction after 2 weeks on trial (all p-values <.05) and in SAS-marital, LIFE-interpersonal, LIFE-satisfaction, Q-LES-Q total, and Q-LES-Q medication satisfaction after 4 weeks on trial (all p-values <.05). The rates of change in depression scores were significantly correlated with the rates of change in psychosocial variables. Coefficients ranged from .250 to .432 for significant SAS subscales, .329 to .543 for significant LIFE-RIFT subscales, and -.323 to -.497 for significant Q-LES-Q ratings. The baseline rating of the LIFE-RIFT interpersonal subscale was significant (WaldChisq(1)=6.17, p=.013) in predicting response to risperidone augmentation while the baseline SAS-social subscale approached significance (WaldChisq(1)=3.43, p=.064) in predicting response to risperidone.
Discussion: For patients with difficult-to-treat depression, augmenting an antidepressant with risperidone has a significant impact on many areas of a patient's psychosocial functioning. Baseline levels of some areas of interpersonal and social functioning may be helpful in predicting a patient's response to risperidone augmentation.

54. A Novel Melatonin Partial Agonist with Sleep-Promoting Properties

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Sponsor: Pierre Blier

Background: Melatonin (MLT, N-acetyl-5-methoxytryptamine) is a neurohormone implicated in several physiopathological conditions, including the regulation of anxiety, mood, sleep and circadian rhythms. These effects of MLT result from the interaction with its G-protein coupled brain receptors, called MT1 and MT2. The function of these receptors has not yet been fully characterized due to the paucity of receptor subtype-selective compounds. Here we propose– for the first time – a novel drug acting as an MT2 receptor partial agonist (called "UCM 765"). UCM765(N-{2-[(3-Methoxyphenyl)-phenylamino]ethyl}acetamide), possesses a better MT2 affinity (pKi = 10.18) than melatonin (pKi = 9.59) and boasts about a 100-fold higher affinity for the human recombinant MT2 (pKi = 10.18) than for the MT1 (pKi = 8.28) subtype expressed in NIH3T3 cells. UCM 765 behaved as melatonin partial agonist, producing a concentration dependent maximal stimulation of basal [(35)S]GTP-gamma-S binding lower than that produced by melatonin. The relative intrinsic activity (IAr) values of UCM 765, obtained by dividing the maximum UCM 765-induced G-protein activation by that of MLT, were the following: IAr-hMT1 = 0.8; IAr-hMT2 = 0.6.
Methods: In order to test the sleep-promoting properties of UCM 765, we performed electroencephalogram (EEG) and electromyogram (EMG) in freely-moving rats (n=7 per group) from 6 PM to 9 PM following a subcutaneous injection of either vehicle (DMSO 70% and saline 30%), diazepam (2 mg/kg) and UCM 765 (40 mg/kg). ANOVA one-way RM was used to compare the three treatment groups.
Results: We evaluated the different stages of sleep and vigilance: awake stage, slow waves sleep (SWS) and the paradoxical sleep (PS), also called rapid eye movement sleep (REM). Rats treated with UCM 765 exhibited a shorter latency to the onset of the first SWS period compared to control (ctrl) (ctrl: 21.56 ± 3.87 min; UCM 765: 11.26 ± 3.32 min, p<0.05) similar to diazepam (12.64 ± 3.59 min). The UCM 765 also increased the duration of the SWS (ctrl: 55.95 ± 3.88 min; UCM 765: 88.78 ± 4.34 min, p<0.05; diazepam: 90.18 ± 6.02, p<0.05), but not the number of episodes of SWS compared to control. Results on PS assessment indicate that UCM 765, similar to diazepam, prolonged the latency of the first episode of PS (ctrl: 49.42 ± 4.43min; UCM 765: 69.59 ± 8.13 min, p<0.05; diazepam: 72.25 ± 7.36, p<0.05), but did not change the duration and quantity of PS episodes.
Discussion: These results suggest that UCM 765 at the dose of 40 mg/kg decreases the latency of the SWS onset, and prolongs SWS duration, but does not change the sleeping architecture. Since reduced PS (REM) latency and a diminished SWS have been observed in patients suffering from depression and sleep disorders, the fact that UCM 765 prolongs PS onset suggests that this drug may also improve the quality of sleep in depressive patients. These characteristics of UCM 765 indicate that it may be a good candidate as hypnotic drug.

55. Electrophysiological Response to an Emotional Go/Nogo Test and Remission of Geriatric Depression

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Background: To assess the relationship of ACC function to change in depressive symptoms during treatment, we studied error-related negativity (ERN) and error positivity (Pe) during an Emotional Go/No-go challenge, a function activating the rostral ACC. ERN and Pe occur when errors are committed. The ERN represents the detection of conflict between incompatible stimuli by the ACC. The Pe occurs during a post-response evaluation of error by the ACC or as an emotional reaction to error. The ERN generator is in medial prefrontal areas, in or very near the dorsal ACC. The Pe generators are in the parietal cortex and the rostral ACC, an area associated with reward and with the assessment of emotional valence. This study tested the hypothesis that ERN and Pe during the Emotional Go/No-go task distinguish depressed elders who remain symptomatic from those who achieve remission after escitalopram treatment.
Methods: 12 patients (>60 years), with major depression (by SCID, DSM-IV) and a Hamilton Depression Rating Scale (HDRS) score of 17 or higher received escitalopram 10 mg for 8 weeks after a 2 week single-blind placebo phase. The Emotional Go/No-Go task consisted of 400 (20 blocks of 20 trials) emotionally valenced words chosen from the University of Florida's normed list. In the first block of trials, subjects were instructed to keypress (Go) only when seeing a pleasant word. In the next block, subjects were instructed to keypress when presented with unpleasant words. The blocks alternated between responding to pleasant and unpleasant words. ERPs were recorded with the 128-channel high-density EGI System 200. ERN was the largest negative deflection within -25 to 150 msec after an incorrect response and Pe was the largest positive deflection within 150-200 msec. Amplitude was measured at Fcz and Cz and a medial-frontal lead group (the mean of Fcz and two adjacent leads). BESA was used to construct topographic maps.
Results: 6 subjects achieved remission (final HDRS<=10) and 6 remained symptomatic. There were no significant differences between remitted and non-remitted subjects in age, baseline depression severity, cognitive impairment, error rates or reaction time during the Go/Nogo task, or dosage of escitalopram. Non-remitters had a larger ERN in Cz (t=2.24, df=10, p<0.049), the median frontal lead group (t=2.95, df=10,p<0.04) and FCz (t=1.61, df=10, p<0.14) than remitters. Moreover, non-remitter had a smaller Pe in Cz (t=2.96, df=10, p<0.01), the median frontal group (t=5.12, df=10, p<0.0001), and FCz (t=5.32, df=10, p<0.0001). These leads were located at the regions with the highest amplitude of ERN and Pe.
Discussion: To our knowledge, this is the first report of a double dissociation in processing error-related functions in depressed patients with different treatment outcomes. Our observation is consistent with findings suggesting that ACC dysfunction is associated with

poor response to antidepressants. Abnormal performance in the Stroop test has been associated with ACC dysfunction and predicts limited response of geriatric depression to citalopram. ACC metabolism and activity is a predictor of antidepressant response. Large left frontal ERN amplitude following Stroop activation predicts limited change in depressive symptoms in geriatric patients treated with citalopram. Finally microstructural white matter abnormalities lateral to ACC may predict poor remission of geriatric depression. Limitations of this study include the small number of subjects, the lack of a placebo arms and limited neuropsychological assessment.

56. Does the Probability of Receiving Placebo Influence the Likelihood of Responding to Placebo or Clinical Trial Outcome? A Meta-Regression of Double-Blind, Randomized Clinical Trials in MDD

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Placebo response rates represent a major obstacle to drug development in major depressive disorder (MDD). Unfortunately, however, there has been very little systematic study on the effects of various elements of study design on placebo response rates and clinical trial outcome. It has previously been reported that a greater degree of expectation of improvement at baseline resulted in a greater probability of subsequently responding to treatment. However, whether this relationship can be attributed to either the "true" antidepressant or placebo response is unclear. In addition, it is equally unclear whether the likelihood of receiving active treatment or placebo, a proxy of the degree of expectation of improvement, may itself influence the probability of responding to active treatment, to placebo, as well as influence clinical trial outcome. Thus, the goal of this work was to examine whether the probability of receiving placebo influences placebo response rates, antidepressant response rates, and clinical trial outcome in major depressive disorder MDD.
Methods: Pubmed/medline were searched for randomized, double-blind, placebo-controlled trials of antidepressants for adults with MDD. The probability of receiving placebo was calculated based on: i) the number and type of treatment arms and, ii) the likelihood of being randomized to each treatment arm. Two separate multiple regressions were conducted with antidepressant response rates or placebo response rates as the dependent variable, and: i) the probability of receiving placebo, ii) year of publication, iii) study duration, and iv) sample size as the independent variables. Finally, a meta-regression was performed, with the odds ratio of responding to antidepressant versus placebo as the dependent variable and: i) the probability of receiving placebo, ii) year of publication, iii) study duration, and iv) sample size as the independent variables.
Results: 139 manuscripts were identified involving 163 separate clinical trials eligible for inclusion in the meta-analysis. These trials involved n=34,780 patients randomized to 248 separate antidepressant-placebo comparisons (n=22,065 and 12,715, respectively). Overall pooled response rates for the two treatment groups were 53.4% and 36.6% (OR=1.448; 95%CI=1.393-1.505, $p<0.0001$ random-effects meta-analysis). The results of the meta-regression suggest that the probability of receiving placebo (coefficient=0.55; p=0.02), sample size (coefficient=-0.008; p=0.013), year of publication (coefficient=-0.01; p=0.001), but not study duration ($p>0.05$) were independent predictors of the odds ratio of responding to antidepressants versus placebo. The results of the multiple regression suggest that the probability of receiving placebo (coefficient=-0.229; p=0.01), year of publication (coefficient=0.005; p=0.0004), but not sample size ($p>0.05$), or study duration ($p>0.05$) were independent predictors of placebo response. Finally, the results of the multiple regression suggest that none of the four factors predicted antidepressant treatment response ($p>0.05$).
Discussion: A lower likelihood of receiving placebo predicted greater placebo response rates and lesser antidepressant-placebo separation at endpoint, but did not influence the likelihood of responding to antidepressants. This was independent of other factors including year of publication, study duration and sample size. Implications on study design will be discussed.

57. Impact of Childhood Abuse on Treatment Outcome in Depression: Results of A Double-Blind Risperidone Augmentation Trial

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Sponsor: Charles Nemeroff

Background: Vulnerability to depression is markedly increased by early life traumatic experiences. Identification of the neurobiological substrates affected by these early life adverse experiences should lead to the development of more effective treatments. Until then, different approaches have been evaluated in patients with a suboptimal response to antidepressants. This post-hoc analysis assessed whether reported childhood abuse (sexual, physical, and/or emotional) impacted clinical response to adjunctive risperidone in patients with major depressive disorder (MDD) suboptimally responsive to antidepressant therapy.
Methods: Data are from a U.S. trial in patients aged 18 to 65 years with MDD and a Clinical Global Impressions of Severity (CGI-S) score ≥4, who had received ≥4 weeks of an antidepressant. During a 4-week open-label phase, patients received their antidepressant at an optimized dose. Those with persistent depression (CGI-S score ≥4 and a Carroll Depression Scale score ≥20) were randomized to 6-weeks of double-blind (DB) risperidone or placebo augmentation to the antidepressant regimen. The investigator-rated 17-item Hamilton Rating Scale for Depression (HRSD-17) and patient-rated Most Troubling Symptoms (MTS) and Quality of Life Enjoyment and Satisfaction Questionnaire (Q-LES-Q) were completed at baseline and at week 6 (last observation carried forward [LOCF]).
Results: Childhood abuse was reported by 53% (141/268) of DB patients—emotional abuse alone was the most frequent (17.5%) followed by any 2 types of abuse (15.3%), 3 types of abuse (13.8%), sexual abuse alone (4.1%), and physical abuse alone (1.9%). Baseline demographic and disease characteristics among those with reported abuse were similar to those without abuse with exceptions of a greater number of years since first episode (p=0.009) and higher HRSD-17 anxiety/somatization (p=0.048) and Maier-Philip severity (p=0.019) subscale scores. HRSD-17, MTS and Q-LES-Q scores improved significantly ($p<0.05$) from baseline to week 6 LOCF in all patients, with the decreases being significantly greater in the risperidone vs. placebo group. No significant differences were observed when changes from baseline in HRSD-17, MTS, and Q-LES-Q were analyzed by abuse status; treatment by abuse status interaction; or between abuse vs. no abuse groups randomized to placebo, risperidone or overall. Percentages of remitters (HRSD-17 ≤7) and responders (HRSD-17 >50% decrease from baseline) were greater with risperidone vs. placebo in those with abuse (19.7% vs. 9.2% and 40.8% vs. 23.1%) and in those with no abuse (19.7% vs. 9.8% and 41% vs. 34.4%).
Discussion: Among all patients, augmentation with risperidone produced improvements vs. placebo in patient- and clinician-rated efficacy measures. This post-hoc analysis did not detect an impact of abuse on response to risperidone augmentation. This is in contrast to prior reports in patients early life trauma and may be the result of inconsistent disclosure of abuse status (i.e., no abuse group may have included patients who did not disclose abuse), a differential impact among types of abuse, or an interaction between abuse and other variables. Further analysis of this database may identify a differential effect among types of abuse or an interaction with other relevant fac-

tors impacting the relationship between childhood abuse and treatment outcome in adults with depression. Supported by funding from Janssen, L.P.

58. Continuation ECT versus Pharmacotherapy for Relapse Prevention in Major Depression: A Multi-Site Study from CORE

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Sponsor: Max Fink

Background: Electroconvulsive therapy (ECT) is an extremely effective acute treatment for major depression but has never been systematically assessed as a strategy for relapse prevention. In a multi-site, NIMH-funded study we evaluated the comparative efficacy of continuation-ECT (C-ECT) and the combination of lithium plus nortriptyline (C-Pharm) for relapse prevention.
Methods: Two hundred one patients with unipolar depression who had remitted with a course of bilateral ECT were randomized to receive C-ECT or C-Pharm for 6 months. C-ECT patients received 10 treatments in a standardized tapered schedule. No psychotropic medications were allowed other than occasional use of lorazepam. C-Pharm patients received Lithium + Nortriptyline and were evaluated at the same intervals. The main outcome measure was depressive relapse defined as HAMD ≥ 16 sustained for 1 week.
Results: At the end of the study 46.1% in the C-ECT group remained remitted, 37.1% relapsed and 16.8% dropped out. In the C-Pharm group 46.3% remained remitted, 31.6% relapsed, and 22.1% dropped out. No statistically significant differences in overall survival curves and time to relapse were found. Mean time to relapse was 9.1 weeks (sd 7.0) for the C-ECT group and 6.7 weeks (sd 4.6) for the pharmacotherapy group (p=0.13). Both groups had relapse proportions significantly lower than an historical placebo control from a similarly designed study.
Discussion: C-ECT is a viable strategy for relapse prevention in unipolar depression after remission with ECT. The Efficacy of C-ECT and the combination of lithium and nortriptyline is similar. C-ECT and the C-Pharm were shown to be superior to an historical placebo control, but still more effective strategies for relapse prevention in depression are needed.

59. Enhancement of Desipramine- and Tranylcypromine-Induced "Antidepressant-Like" Effects on DRL 72-s Behavior By the Selective 5-HT$_{2A}$ Receptor Antagonist M100907

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Sponsor: Darryle D. Schoepp

Background: The common pharmacological factor between augmentation of selective serotonin reuptake inhibitors (SSRIs) with either mianserin, mirtazapine, olanzapine or risperidone is potent blockade of 5-HT2A receptors (Marek et al., 2003). Consistent with this hypothesis, we previously reported that low doses of the selective 5-HT2A receptor antagonist M100907 enhanced antidepressant-like effects (increased reinforcement rate, decreased response rate and a rightward shift in the interresponse time (IRT) distribution) of the SSRI fluoxetine when administered to rats performing on the differential-reinforcement-of-low rate 72-s (DRL 72-s) schedule (Marek et al., 2005).
Methods: The present studies were designed to extend the specificity of these findings by combining administration of M100907 with the tricyclic antidepressant desipramine (DMI), the monoamine oxidase inhibitor (MAOI) tranylcypromine, and the typical antipsychotic drug haloperidol. Male Sprague-Dawley rats were water-deprived except for a 20 min. period of water availability after each daily behavioral session. These rats were first trained to lever press on a continuous reinforcement schedule. After learning to lever press, the animals were shifted first to a DRL 18-s schedule and then a DRL 72-s schedule until their behavior had stabilized.
Results: A submaximal concentration of M100907(0.00625 mg/kg, ip, 1 hr pretreatment) potentiated the reinforcement rate-increasing effect of tranylcypromine (1.25 mg/kg, ip) and desipramine (2.5 mg/kg, ip). We performed a similar experiment with haloperidol and M100907. While there was a trend towards an increased reinforcement rate with M100907 (0.0125 mg/kg, ip), there was neither a significant effect of haloperidol alone (0.04-0.16 mg/kg, ip) nor the interaction of these drugs on the reinforcement rate. The only significant effect on the response rate was an expected decrease induced by haloperidol itself.
Discussion: These results are consistent with the hypothesis that blockade of the 5-HT$_{2A}$ receptor and attenuation of "impulsive" responding mediates, at least in part, an optimal antidepressant-like effect in rats performing on a DRL 72-s schedule. (The present work was conducted at the Yale School of Medicine (Dept. of Psychiatry) and were funded by NIH, NARSAD and the State of Connecticut).

60. Riluzole Blocks Stress-Related Effects on Behavior and Enhances Glutamate/Glutamine Cycle Flux

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Background: Increasing evidence supports a relationship between stress, the glutamatergic system and depression (Sapolsky 2000). Extracellular glutamate concentrations are increased in several brain regions including prefrontal cortex and hippocampus following exposure to behavioral stressors. Recent studies demonstrate that several classes of agents known to regulate glutamate neurotransmission possess antidepressant-like properties in animal models, as well as in patients diagnosed with mood disorders. Riluzole is a FDA-approved glutamate-modulating agent, successfully used to delay ALS progression in patients and the onset of disease in animal models (Doble 1999, Jackson et al 2002). Riluzole is believed to modulate glutamatergic neurotransmission via effects of neuronal glutamate release and enhancement of glial glutamate uptake. Several recent open label studies suggest a therapeutic action of riluzole in patients experiencing major depressive episodes (Zarate et al 2003,2005 Sanacora et al 2004,2006). Yet, little preclinical work has been done to examine the effects of the drug in animal models of depression, or to explore the potential mechanism(s) of antidepressant action associated with the drug. The aim of this study is to characterize the antidepressant-like properties of riluzole in a rodent model, and to examine the effects of the drug on glutamate neurotransmission.
Methods: We assessed the effect of riluzole in the chronic unpredictable stress (CUS) paradigm, using measures of despair (active avoidance) and anhedonia (sucrose preference) as the primary behavioral outcome measures. The CUS procedure consisted of Sprague Dawley rats receiving 12 different and randomized stressors (2 stressors/day) for 35 days. Riluzole (4mg/kg, i.p), fluoxetine (5mg/kg, i.p.) or saline were administrated once daily during the last 3 weeks of CUS to simulate a realistic course of antidepressant intervention. The effect of riluzole on neuronal metabolism and glutamate/glutamine cycling was explored using 13C-MRS. Animals were infused with [1-13C]glucose (0.75umol/100g/min) via tail vein infusion for 10 minutes prior to sacrifice. Total and 13C enriched concentrations of amino acids in the frontal cortex were determined by ex vivo MRS studies at 11.7T.
Results: Chronic treatment with riluzole reversed the CUS effects on despair and anhedonia. Riluzole prevented the CUS-induced

deficit in the active avoidance test, a measure of despair or helplessness, after 14 days of treatment (P<0.05). CUS induced an anhedonic state indexed by sucrose preference test (P<0.05) that was first observed after 2 weeks and was maintained at the end of the 35day period for the saline treated animals (P<0.01). Chronic riluzole treatment for twenty-one days reversed the effect of CUS on the anhedonic state (P<0.05). A similar effect was found with chronic fluoxetine treatment. Frontal cortex concentrations of 13C enriched glutamate (Glu-C4), GABA (GABA-C2), and glutamine (Gln-C4) were all increased in the animals receiving chronic riluzole (P< 0.001, P< 0.5, P< 0.01, respectively). However, no change in total frontal cortex glutamate, GABA, and glutamine content was observed.
Discussion: The results demonstrate that riluzole is effective in preventing and reversing the stress-induced depression-like behaviors of despair and anhedonia. In addition, based on findings of increased 13C labeling of the amino acids there is preliminary evidence that chronic administration of riluzole is associated with increased glutamate/glutamine cyling in the frontal cortex. These data are consistent with the hypothesis that stress-related changes in the regulation glutamate cycling and amino acid neurotransmission are associated with pathophysiology and possibly pathogenesis of mood and anxiety disorders. Supported by MH076222, MH25642 and MH45481.

61. Decreased Protein Kinase C (PKC) in Platelets of Pediatric Bipolar Patients: Effect of Treatment with Mood Stabilizing Drugs
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Background: Pediatric Bipolar Disorders (PBD) is a major public health concern with poor recovery between episodes and a high relapses rate. Whereas there is some understanding of the neurobiological abnormalities associated with adult bipolar illness very little is known about the biological abnormalities associated with PBD. Previous studies with the platelets and postmortem brain of adult bipolar disorder patients suggest abnormalities of certain monoamine receptors and receptor-linked signaling system. It has been also found that protein kinase C (PKC), which is one of the components of the phosphoinositide (PI) signaling system, is not only abnormal in bipolar illness but is also affected by treatment with mood stabilizing drugs such as lithium (Li).
Methods: We have therefore studied the role of PKC in pediatric bipolar illness by determining protein expression of several PKC isozymes as well PKC activity in the platelets obtained from PBD patients during a drug-free period and after 8 weeks of treatment with mood stabilizing drugs. Patients were recruited in the Pediatric Mood Disorders Clinic of the University of Illinois at Chicago and were drug-free for at least one week before the blood was drawn. Patients were diagnosed according to DSM-IV criteria using the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS). Manic symptoms were rated using Young Mania Rating Scale (YMRS). Matched normal controls were also drug-free at the time of the study. PKC activity and expression of PKC α, PKC βI, PKC βII, PKC δ were determined using Western blot technique in platelets of 23 drug-free and 23 normal control subjects. The YMRS score was 25 prior to treatment and dropped down to 6.44 after 8 weeks of treatment.
Results: We observed a significant decrease in PKC activity in membrane and cytosol fractions of platelets obtained from PBD patients compared with normal control subjects. When we determined the protein expression level of various PKC isozymes, we found that the protein expression levels of PKC βI and PKC βII was significantly decreased in both membrane and cytosol fractions of PBD patients compared to normal control subjects. On the other hand, there was no significant difference in the protein expression levels of either PKC α or PKC δ between PBD patients and normal control subjects. Treatment with mood stabilizing drugs for 8 weeks, including Li and Valproic acid, caused a significant increase in the PKC activity in 16 patients compared with the baseline levels and was almost similar to the levels in the normal control subjects. On the other hand, 8 weeks of treatment with mood stabilizing drugs did not cause any significant changes in the expression of any of the PKC isozymes compared to the baseline levels.
Discussion: These studies suggested that PBD is associated with a decrease in PKC activity and protein expression of PKC βI and PCK βII in the platelets of bipolar patients compared with normal control subjects and treatment with mood stabilizing drugs normalizes the abnormality of PKC activity but not of the protein expressions of PKC βI and PKC βII in these patients. PKC may thus play a significant role in the pathophysiology of PBD and in the therapeutic mechanism of action of mood stabilizing drugs. Support contributed by: NIMH grant RO1 MH56528 (G. Pandey)

62. Effects of Light Deprivation on Brain GABA and Glutamate in Healthy and Depressed Individuals
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Background: Reductions in occipital brain GABA and elevations in occipital brain glutamate have been reported in patients with major depressive disorder (MDD). The changes might be associated with reduced glutamatergic drive or with impaired astrocytic function. One major excitatory drive for the occipital cortex is the glutamatergic projection from the lateral geniculate nucleus of the thalamus. Light deprivation attenuates geniculo-cortical activity, reducing excitation in the visual cortex, so in this study we attempted to reproduce the GABA and glutamate findings in healthy and depressed individuals by using light deprivation to decrease excitatory drive of the visual cortex.
Methods: GABA and glutamate+glutamine (Glx) were measured in 7 healthy subjects, using 1H magnetic resonance spectroscopy (MRS) in a 3x1.5x3 cm3 occipital volume with a 2.1T magnet (n = 5) and after an upgrade, at 4T (n = 2). MRS was done for 20 minutes with the lights on and then 40 minutes in total darkness. Nine patients diagnosed with MDD, confirmed by SCID interview, were studied in the same way (4 at 2.1T magnet and 5 at 4T). In four additional experiments, MRS was performed over the same period with the lights on.
Results: Healthy subjects' occipital GABA decreased 28±15% (mean ± SD) by 45 minutes in the dark (Table 1) but only 1% in the light. The average Glx showed an increase of 5-10%, but with p > 0.1, any change was inconclusive and certainly no different from what is seen in with continuous ambient light (Table 2). Table 2 also shows that with ambient light, GABA does not fall in healthy subjects. Patients' initial occipital GABA did not differ from the controls' and it decreased on average, but the variability in the response rendered any changes insignificant (Table 3). No changes were observed for Glx. GABA in depressed subjects showed more heterogeneous responses to darkness than did the healthy subjects, with GABA in 7/9 falling to between 50 and 82% of the starting value and 2/9 showing no GABAergic response to the dark. The variability in the responses showed no relationship to the starting concentration of brain GABA.
Discussion: Healthy subjects showed a robust decrease of GABA in the occipital cortex in the dark. Low glutamatergic drive that occurs with light deprivation may be able to reduce occipital GABA, but with variability in MDD that depends on pathophysiology. **Acknowledgements:**NARSAD (GFM), K02AA13430(GFM), CNPq (RG), Donaghue Foundation (GS).

63. Converging Pharmacological, Microstimulation, and Physiological Evidence that the Prelimbic Prefrontal Cortex Mediates Expression of Learned Fears in the Rat

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Background: Previous studies in rats have implicated the infralimbic (IL) subregion of the medial prefrontal cortex (mPFC) in the recall of fear extinction, suggesting that this area inhibits fear expression. Here, we examined the role of the adjacent prelimbic (PL) subregion of mPFC using similar techniques to those we employed in investigations of IL.

Methods: Rats were prepared for either: 1) inactivation of PL via local infusion of Na channel blocker tetrodotoxin (TTX; 5ng in 0.3uL) through surgically implanted cannulas, 2) microstimulation of PL through surgically implanted bipolar stimulating electrodes, or 3) monitoring of PL extracellular action potentials via surgically implanted microdrives.

Results: Pharmacological inactivation: We infused TTX into PL prior to exposing rats to three fear-inducing stimuli: a tone CS, unsignaled footshock, and a live cat. PL inactivation substantially reduced tone-induced and shock-induced freezing (both conditioned fears). Freezing in TTX-infused rats was only 20% of saline-infused controls ($p < 0.001$). In contrast, TTX had no effect on freezing to the cat (an unconditioned fear, $p=0.62$). Thus, PL activity is necessary for the expression of learned, but not unlearned, fear. Microstimulation: To determine if activation of PL might increase fear, we used microstimulation of PL. On day 1, rats received 5 tone-shock pairings. On day 2, rats received tones paired with PL microstimulation (100 uA, 100Hz, 100-400 ms after tone onset), or tones without microstimulation. Microstimulation of PL increased the expression of conditioned fear and impaired extinction. Thus, in agreement with inactivation findings, activation of PL increases the expression of conditioned fear. Unit-recording: Prior studies indicate that PL neurons signal conditioned fear associations. To determine if conditioned activity of PL neurons was correlated with freezing behavior, we recorded the activity of single PL neurons during learning. Rats received five tones (30 sec; 4 kHz), each paired with a footshock (0.3 mA, 0.5 sec). Two hours after conditioning, rats were tested for fear to the tone. Prior to training, there was little tone responsiveness in PL neurons. After conditioning, approximately 20% of PL cells showed sustained tone responses, many of which outlasted the tone. Freezing was assessed in 3-sec bins prior to, during, and after the tone. Conditioned PL tone responses were significantly correlated with the time course of freezing to the tone ($r= 0.82$; $p<0.001$), consistent with a role in expression of fear.

Discussion: Taken together, these findings suggest that activation of PL is a pre-requisite for expression of conditioned fear, and that activation of the amygdala alone is not sufficient. Sustained activity in PL neurons could support freezing via excitatory projections to the basal nucleus of the amygdala, a structure critical for fear expression. An important translational goal is to identify the human homologue of PL, which could be a target for future therapies for anxiety disorders. An interesting parallel line of investigation suggests that the homologue of PL may be the dorsal anterior cingulate cortex (see Milad et al., this meeting).

64. Stress Results in Region Specific Decreases in Expression of Angiogenic Factors in the Brain

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Cerebral hypoperfusion is a characteristic of neurodegenerative diseases and psychiatric disorders including Alzheimer's disease, depression, alcoholism, and substance abuse. Hypoperfusion impairs nutrient and oxygen delivery to neural tissue and can result in cellular edema, gliosis and perivascular inflammatory infiltrate. Changes in angiogenesis and thereby changes in perfusion of neural tissue, may alter synaptic plasticity. Furthermore, a causal interaction may exist between hormone regulated angiogenesis and neurogenesis in the brain. Exposure to chronic stress alters neuronal cytoarchitecture including decreases in hippocampal dendrite complexity and inhibition of neurogenesis. However, little is known about the effects of chronic stress on the vasculature of the brain. Given the crucial role of neuronal vasculature in the maintenance of neural tissue, an understanding of the effects of chronic stress on brain vasculature may provide insights into the mechanisms that underlie stress-evoked changes in neural plasticity. The current study tested the hypothesis that exposure to prenatal glucocorticoids augments stress-induced changes in cerebral angiogenesis in the adult rat.

Methods: Pregnant Long Evans rats were administered a subcutaneous injection of either dexamethasone (DEX; 100 µg/kg in 0.1 ml sterile saline; n = 8), or saline (0.1 ml; n = 8) daily during the last week of gestation. At 90 days of age, offspring were individually housed and assigned to either a handled group (30 s handling/day; n = 8 DEX offsping, n = 8 saline offspring) or restraint group (2 hr/day for 14 days; n = 8 DEX offspring, n = 8 saline offspring). This duration of restraint was chosen because 10 days of exposure is sufficient to alter neuronal architecture and changes in vasculature can take up to 2 wks to be detectable. Immediately following the 14th exposure, rats were either rapidly decapitated for collection of brain tissue for qRT-PCR or perfused for histological analysis of vessel density. qRT-PCR was conducted for expression of mRNA for VEGF, angiopoietin 1, angiopoietin 2, and Tie-2 and expression levels were compared to the house keeping gene Arbp. For histological analysis of vessel density, brains were cryoprotected in sucrose and then cut on a cryostat. Immunohistochemistry for von Willebrand factor was conducted and qdots were used for visualization.

Results: Expression levels of angiogenic factors suggested that exposure to restraint as an adult produced a destabilization of capillaries and potentially vessel retraction in the prefrontal cortex and hypothalamus. The combination of prenatal DEX and exposure to restraint as an adult, but not restraint alone, resulted in angiogenic factor expression that suggested destabilization of capillaries and vessel retraction in the hippocampus. The opposite effect was present in the amygdala such that the combination of prenatal DEX and adult exposure to restraint resulted in an expression profile that suggested angiogenesis may occur. Histological analysis of vessel density will provide insight into the physiological relevance of the documented changes in angiogenic factor gene expression profiles.

Discussion: The data presented here are the first to demonstrate a change in angiogenesis in the hypothalamus, prefrontal cortex, and amygdala following stress exposure. Futhermore, prenatal exposure to glucocorticoids exacerbates stress-induced changes in cerebral angiogenesis. Given the intimate interactions between cerebral vasculature and neurons, changes in cerebral vasculature could contribute to post-stress neuronal pathology. Insight into these relationships may provide additional avenues for treatment of stress-induced neuronal pathology.

65. New Approaches to Modeling Affective Disorders, Different Facets, Strains, Species, Individuals and Molecules

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: The lack of appropriate animal models is a major limitation in research of affective disorders. Previous attempts to develop

comprehensive models for the disorder did not culminate to validated and practical models.
Methods: We have therefore chosen to explore a number of other approaches to develop new and better models including: Modeling facets of the disorders. Modeling based on strain differences. Modeling based on species differences. Modeling based on individual differences. Modeling based on tentatively involved mechanisms. Whereas these attempts are based on different approaches, they are clearly not mutually exclusive and can serve together to support the effort to gain better understanding of the underlying biology of affective disorders and for the development and screening of new potential therapies.
Results: Experiments testing some of the above mentioned approaches already resulted in new potential models that are currently further developed: Modeling facets of the diseases – a simplified resident-intruder paradigm was validated as a possible model for the aggression facet of mania. Preliminary data support brief stress induced reduction in anxiety as a possible model for the risk-taking behavior facet of mania. Strain Differences – Black Swiss mice demonstrate a set of behaviors that compared with other strains appear to be manic-like. These behaviors include increased activity, increased hedonia, decreased anxiety (or increased risk taking) and increased response to psychostimulants. These behaviors are ameliorated by chronic treatment with mood stabilizers. Species differences – the fat sand rat is a diurnal rodent. We have shown that when these animals are exposed to short day light cycle they develop depression-like behaviors compared with control animal with long light days. It is suggested that the fat sand rat may be a potential species to model seasonal affective disorder.
Discussion: It is our contention that a variety of approaches must be taken in order to develop a set of appropriate, validated and practical animal models for affective disorders.

66. Behavioral Effects of Antidepressant Drugs in Beta Adrenergic Receptor Knockout Mice Responding under a Differential-Reinforcement-of-Low-Rate (DRL) Schedule

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Sponsor: James M. O'Donnell

Background: Beta adrenergic receptors have been implicated in the mediation of the behavioral effects of antidepressant, but their actions have not been fully elucidated.
Methods: Effects of the antidepressants desipramine, fluoxetine, and rolipram, selective inhibitors of norepinephrine reuptake, serotonin reuptake, and phosphodiesterase-4 (PDE4), respectively, were determined in mice under a differential-reinforcement-of-low-rate (DRL) 36-sec schedule.
Results: In wild-type mice, administration of either desipramine (3-50 mg/kg) or rolipram (0.1-3 mg/kg) produced antidepressant-like effects on DRL behavior, i.e., decreased response rates and increased reinforcement rates. In contrast, fluoxetine (3-30 mg/kg) only decreased response rates. In mice deficient in beta-2 or both beta-1 and beta-2 adrenergic receptors, desipramine produced antidepressant-like effects similar to those in wild-type controls. In contrast, in mice deficient in beta-1 adrenergic receptors, desipramine did not alter DRL behavior except at the highest dose of 50 mg/kg, which decreased both response and reinforcement rates. In all the gene knockout mice, rolipram only decreased response rates, whereas fluoxetine did not alter DRL behavior except at the higher dose (30 mg/kg), which decreased both response and reinforcement rates.
Discussion: 1) While the effect of desipramine was not dependent on beta-2 adrenergic receptors, beta-1 adrenergic receptors appear to play a role in the mediation of the antidepressant-like effect of desipramine on DRL behavior. This is consistent with our previous findings that beta-1 adrenergic receptors are important for antidepressant-like actions. 2) The antidepressant-like effect of rolipram was attenuated in beta adrenergic receptor knockout mice relative to their wild-type controls, suggesting that both beta-1 and beta-2 adrenergic receptors are involved, at least partially, in the effect of rolipram on DRL behavior. 3) Given that fluoxetine did not produce a typical antidepressant-like effect on DRL behavior even if in wild-type mice, it may not be sensitive to the effects of serotonin reuptake inhibitor without some modification, as also proved necessary for the forced swim test (Supported by research grants from NIMH).

67. Dopamine and Serotonine Transporter Genes in Geriatric Depression: Clinincal Features and Treatment Response

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Sponsor: Gwenn Smith

Background: The authors examined the role of dopamine and serotonin transporter genetic polymorphisms in clinical and cognitive features of subjects with late-life depression, and in preferential treatment response to the combination of methylphenidate and citalopram.
Methods: The authors studied fifteen outpatients with current episodes of non-psychotic major depression in a ten-week double-blind trial of methylphenidate combined with citalopram and compared to citalopram and placebo. Response was defined as a score on the Hamilton Depression Rating Scale (24-item) of less than 10. All underwent genotyping to determine the dopamine (DAT VNTR) and serotonin (5HTTLPR) transporters' polymorphisms, as well as comprehensive neuropsychological and neuropsychiatric assessments.
Results: Subjects homozygous by DAT VNTR-10 genotype had greater cognitive executive impairment at baseline compared to others. However, they responded preferentially to methylphenidate added to citalopram with a greater reduction in depression severity over time and improvement in cognitive tests of executive function compared to other subjects. 5-HTTLPR l-allele carriers had a faster onset of antidepressant response than non-carriers, but no difference in the overall response or cognitive improvement with treatment.
Discussion: DAT VNTR 10/10 genotype may be associated with an endophenotype of late-life depression with executive dysfunction that responds preferentially to methylphenidate added to a selective serotonin reuptake inhibitor, but 5 HTTLPR-l allele might be related to the speed of response, which warrants replication in a large sample.

68. Hippocampal Volume Reduction and HPA-system Activity in Major Depression

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Sponsor: Dirk Hellhammer

Background: It is well known that major depression is frequently accompanied by hypercortisolemia. These elevated glucocorticoid concentrations are thought to be driven by an increased secretion of hypothalamic CRH. There is also ample evidence, mainly from animal studies, suggesting that elevated levels of glucocorticoids may render hippocampal cells more vulnerable ("neuroendangerment") to insults caused by hypoxemia, hypoglycemia, or excitatory aminoacids. Since patients with depression have elevated cortisol levels in addition to clinically relevant cognitive dysfunctions, structural imaging studies investigating hippocampal volumes in patients have yielded mixed results. We hypothesized that their hippocampi are marked by diminished size.
Methods: 24 unipolar depressed in-patients and 14 healthy controls carefully matched for age, gender, and years of education underwent

quantitative 3D magnetic resonance imaging (high-resolution mprage). Saliva cortisol was measured at 0800 and 1600 hrs in patients during a one-week wash-out and the following 4 weeks.
Results: Hippocampal volumes were significantly reduced in the patient group even after adjusting for intracranial brain volume (ICV) and age (residualization). Across groups, age was significantly negatively correlated with uncorrected hippocampal volumes. In patients, severity of disease (baseline HAMD scores) and baseline cortisol levels were not related to hippocampal volumes. However, there was a negative association between duration of the index episode before hospitalization and hippocampal volumes. Additionally, hippocampal volumes were significantly negatively correlated with duration of illness. Finally, we observed a trend for higher hippocampal volumes in those patients who showed a subsequent decrease in cortisol levels under pharmacotherapy.
Discussion: In conclusion, our vigorous methodological approach with distinct volume corrections adds to accumulating evidence of a negative association between depression and hippocampal volume, irrespective of age and gender. Most likely, hyperactivity of the HPA-system contributes towards hippocampal volume loss. Additionally, it is interesting to note that we also observed a trend for a higher number of depressive episodes in patients with smaller hippocampal volumes. This could be suggesting that hippocampal volume either is a result of cumulative insults or it could also be that patients with smaller hippocampi are more prone to relapses of their affective illness.

69. The Efficacy of Second-Generation Antidepressants in Late Life Depression: A Meta-Analysis

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Background: Depression is common in late life. The current meta-analysis was undertaken to assess the clinical trial evidence base for the efficacy of second-generation antidepressants in late life depression.
Methods: The Cochrane Library and other electronic databases were searched for trials. The selection criteria were 1) double-blind, randomized, placebo-controlled trials of second-generation antidepressants; 2) patients 60 years and older living in the community; 3) non-psychotic Major Depression by DSM criteria; and 4) not restricted to a specific medical disorder. Second generation agents were defined as non-tricyclic antidepressants. Two authors identified trials meeting these criteria and extracted the data. Outcome was assessed in the ITT/LOCF samples using 50% improvement on the HAMD or MADRS scale to define response or using a scale-based definition of remission. If the required data was not included in the publication, the data was obtained from the sponsor or investigator. Outcomes were expressed as odds ratios with 95% CIs using Review Manager 4.2 (Cochrane, Oxford, UK).
Results: 22 RCTs were initially identified. 14 studies were eliminated for including patients < 60 years, patients with diagnoses other than MDD, inpatients or nursing home patients, or patients with a specific medical disorder. The 8 studies identified varied in length from 6 to 12 weeks and were performed by the manufacturer of the antidepressant. Seven of the 8 were reported since 2003. All were of good and similar quality. ITT/LOCF response and remission rates were reported in all studies or were obtained from the sponsor or investigator. A total of 1844 patients received active drug and 1410 received placebo. Response rates for the antidepressants ranged from 35% to 68.9% and placebo rates ranged from 18.6% to 47.2%. The odds ratio by meta-analysis comparing response to drugs vs placebo was 1.38 (95%CI 1.19-1.60, z=4.24, p=0.0001). The number needed to treat was 11.5. Remission rates were also significantly higher for drugs than placebo (OR=1.29, CI 1.10-1.51, z=3.09, p=0.002). Seven studies included an SSRI in at least one of the arms. Response rates were higher for SSRIs than placebo (OR=1.33, CI 1.14-1.56, z=3.61, p=0.0003). All of these analyses demonstrated significant heterogeneity among the studies.
Discussion: While the number of randomized controlled trials of antidepressants in late life depression is limited, the evidence to date indicates antidepressants are more effective than placebo in older patients.

70. Dietary n-3 Polyunsaturated Fatty Acid Deprivation in Rats for 15 Weeks Decreases Frontal Cortex Phosphorylated CREB, p38 MAPK Activity and BDNF Expression

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Sponsor: Stanley I. Rapoport

Background: Docosahexaenoic acid (DHA 22:6n-3) is an n-3 polyunsaturated fatty acid (PUFA) that regulates brain structure, signaling and plasticity. Decreased brain DHA concentrations and brain derived neurotrophic factor (BDNF) levels are implicated in bipolar disorder.
Methods: We determined expression of BDNF, CREB DNA binding activity, protein kinase activities and levels of DHA in the frontal cortex of rats placed for 15 weeks after weaning on an n-3 PUFA adequate or deprived diet.
Results: The mean frontal cortex DHA concentration was 28% lower in n-3 PUFA deprived than adequate rats. BDNF mRNA and protein, as well as the DNA binding activity and phosphorylation (Ser-133) of its major transcription factor, cAMP response element binding protein (CREB), were decreased. The brain activity of p38 mitogen activated protein kinase (MAPK), which can phosphorylate CREB at Ser-133, also was reduced, whereas other kinases that can phosphorylate CREB (Ca2+-calmodulin kinase IV, protein kinase C, protein kinase A and mitogen activated protein kinase p44) were not significantly changed by deprivation. The addition of DHA to rat primary cortical astrocytes in vitro increased the BDNF protein level, but induction could be blocked by also adding a p38 MAPK inhibitor.
Discussion: The ability of dietary n-3 PUFA deprivation to downregulate phosphorylated CREB and BDNF via a p38 MAPK-dependent process suggests a mechanisms whereby dietary n-3 PUFA supplementation might be helpful in treating bipolar disorder.Refs: Demar JC, Jr. et al. Lipid Res 47:172-180, 2006; Rao JS et al Mol Psychiatry. In press

71. A Cox-2 Inhibitor (celecoxib) as a Possible Adjunctive Agent to Expedite Treatment Response in Bipolar Depression

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Background: Lithium and valproate inhibit cyclooxygenase-2 (cox-2), an enzyme present in cell membranes, including neurons, and involved in the immune inflammatory response. Cox-2 inhibitors also may protect against the neurotoxicity promoted by glutamate. We investigated the effects of a cox-2 inhibitor (celecoxib) as an adjunctive treatment for bipolar disorders patients who did not show satisfactory response to treatment with mood stabilizers. We hypothesized that the celecoxib treatment would cause greater amelioration of depressive symptoms than a matched placebo.
Methods: Twenty seven bipolar disorder patients, in a depressive or mixed episode according to DSM-IV criteria, and on a stable dose of a mood stabilizer or atypical antipsychotic medication, were randomized to receive 6 weeks of double-blind placebo or celecoxib (400 mg/d) treatment. Current mood stabilizer or antipsychotic medication was kept at the same doses during the trial. Depressive and manic symptoms were measured by the Hamilton Depression Rating Scale (HAM-D) and Young Mania Rating Scale (YMRS), respectively, and side-effects by the UKU Side Effects Rating Scale.
Results: The patients receiving celecoxib showed significantly lower HAM-D scores after one week of treatment compared to the patients

receiving placebo (intent-to-treat analysis, $F(1,25) = 4.7$, $p = 0.039$). However, the two groups did not differ significantly on depressive or manic symptoms from the 2nd week until the end of the trial (figure). The rapid onset of improvement in the first week of treatment also was present when the analysis included only the subjects who completed the full 6 week-trial (11 in each group). Two patients receiving celecoxib dropped out of the study due to side-effects (rash). Reduced salivation, nausea/vomiting and dizziness were statistically more frequent in the celecoxib group.
Discussion: Adjunctive treatment with celecoxib may produce a rapid-onset antidepressant effect in depressive or mixed episodes in bipolar disorder patients. Further research investigating the involvement of the cox-2 pathway in the pathophysiology of bipolar depression and the potential antidepressant and mood stabilizing effects of cox-2 inhibitors are needed.

72. Cortisol and Memory in Childhood Sexual Abuse-Related Posttraumatic Stress Disorder

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Background: High levels of glucocorticoids (cortisol) released during stress have been hypothesized to cause stress-induced hippocampal damage and associated memory deficits, and glucocorticoid administration within the physiological range results in a transient deficit in declarative memory in normal human subjects. We previously reported lower afternoon cortisol concentrations (12-8 pm) in women with abuse-related PTSD. The purpose of this study was to assess the relationship between cortisol and verbal declarative memory function in patients with childhood sexual abuse-related PTSD and comparison subjects.
Methods: Thirty-eight women with and without a history of early childhood sexual abuse and PTSD underwent neuropsychological testing for measurement of verbal declarative memory function on the same day that they underwent measurement of plasma cortisol over a 24-hour diurnal period.
Results: Verbal declarative immediate memory as measured by the Wechsler Memory Scale (WMSL-I) was negatively correlated with afternoon cortisol in the non-PTSD women combined ($r=-0.57$; $df=14$; $p=0.026$) while there was no correlation in the PTSD women ($r=-0.08$; $df=12$; $p=0.79$). The correlation between memory and cortisol was also seen for the overall 24 hour period in the non-PTSD women ($r=-0.50$; $df=14$; $p=0.056$). Diurnal cortisol (measured as the mean AUC over 24 hours) was positively correlated with verbal (but not performance) IQ in the abused PTSD subjects ($r=0.64$; $df=16$; $p=0.005$), but not in non-abused non-PTSD ($r=-0.17$; $df=12$; $p=0.59$) or abused non-PTSD ($r=0.15$; $df=7$; $p=0.71$) women. There was also a correlation between verbal IQ and cortisol for afternoon (12-8 pm) cortisol levels ($r=0.50$; $df=12$; $p=0.04$).
Discussion: These findings suggest that cortisol elevations within the normal pattern of diurnal variability are associated with relative impairment in verbal declarative memory function in normal women, confirming the negative correlation between cortisol and verbal memory function. However PTSD is associated with a loss of the relationship between cortisol levels and memory function.

73. fMRI Brain Activation Differences Among Tasks in Frequently Relapsing Bipolar Patients Later Treated with Long-Acting Injectable Risperidone

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Sponsor: Stephen M. Strakowski

Background: The treatment of frequently relapsing bipolar disorder patients is difficult, and uncertainty about the neurophysiological effects of interventions further complicates treatment. Atypical antipsychotics, including Risperidone, have been shown to be effective mood stabilizers in bipolar disorder. The long-acting injectable form of risperidone (Risperdal Consta®) may prevent frequent relapses by reducing inconsistent medication adherence. In order to identify the effects of subsequent treatment we compared baseline fMRI brain activation patterns on two tasks between healthy and frequently relapsing bipolar patients who were later treated with Risperdal Consta®.
Methods: Seventeen bipolar patients were compared to healthy controls during fMRI scans obtained as subjects engaged in two continuous performance tasks (CPT) at study baseline. MRI data was acquired with 4T Varian INOVA systems at U.C. and McLean Hospital. We collected standard gradient echo EPI images for fMRI and processed the data using event-related and block-design methods in AFNI. The identical-pairs (IP) and degraded-stimulus (DS) versions of the CPT were used. Behavioral data was also collected, and reaction times for hits (true positives) and false alarms (false positives) as well as discriminability and bias measures were calculated.
Results: There were few behavioral differences in task performance between groups on either task at the baseline visit. Neither RT measures nor bias differed between groups in either task. Bipolar patients had significantly poorer performance on the CPT-IP task than the healthy subjects (0.962 versus 0.907 on discriminability scores, $p<0.01$), but no difference in discriminability was evident for the CPT-DS. For the brain activation data bipolar patients exhibited significantly more activation during performance of both tasks than controls in anterior limbic network regions including amygdala, BA 10, insula and anterior cingulate cortex (ACC) as well as superior parietal lobule, dorsolateral prefrontal cortex, and premotor areas. By contrast, healthy subjects only exhibited more activation than bipolars in the CPT-DS in striatum, pregenual ACC, and occipital visual association cortex.
Discussion: Baseline differences in behavioral performance are consistent with the presence of several euthymic patients in the patient population at baseline. Baseline differences in brain activation on the CPT-IP and CPT-DS identify increased activation of anterior limbic network and superior attentional regions, whereas CPT-DS activation differences indicate more activation by healthy controls in striatal, occipital and pregenual regions. The largely visual short-term memory demands of the CPT-IP versus the more challenging object recognition memory demands of the CPT-DS could explain why healthy subjects activate more of the medial prefrontal circuits in during the CPT-DS task.

74. Anhedonia: Is There a Difference Between Interest and Pleasure?

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Sponsor: Fridolin Sulser

Background: Anhedonia has been considered a core symptom of depression since the term was coined by Ribot in 1896. The hedonic response has most commonly included two phases of response: interest and pleasure. In 1965 Klein published his concept of endogenomorphic depression in which he distinguished the two types of pleasure response blunted by depression: appetitive (the pleasure of the chase) and consummatory (the pleasure of the feast). The distinction between interest and pleasure as separate measures of hedonic capacity has not been incorporated into commonly used ratings scales and they are combined into a single anhedonia item in the DSM-IV. Although the distinction makes common sense, there is no empirical investigation into the independence of interest and pleasure as measures of anhedonia in a depressed population. The validation study of the DID Anhedonia Rating Scale (DARS) provided an opportunity to evaluate this independence.

Methods: The DARS departed from previous anhedonia rating scales by dividing pleasurable experiences into eight domains plus a global assessment item. Within each domain interest and pleasure were assessed separately. A semi-structured interview with suggested probe questions was provided to the interviewers and the answers were scored using a GRID format with both the frequency and intensity of each symptom considered in generating the measure of severity. The standard measure of severity used for comparison was the Bech subscale of the Hamilton Depression Rating Scale which was administered after the anhedonia scale. Ninety-four subjects with current or past major depressive disorder without other significant psychopathology were assessed at 5 research sites by trained raters.
Results: The correlations of the scores for the interest and pleasure items were > 0.81 for 7 of the 8 domains of anhedonia; Accomplishment had a correlation of 0.73. Two items traditionally associated with both appetitive and consumatory behavior, sex and appetite for food, had high correlations between interest and pleasure (0.91 and 0.84). Interest and pleasure as measured by a global anhedonia assessment scale had a correlation of 0.91.
Discussion: An analysis of the DARS validation study data shows that response to items probing interest in hedonic activities correlated highly with responses to items asking about the pleasure derived from those activities. From an assessment point of view, these data indicate that one can ask either about interest or pleasure. However, questions asking about pleasure suffer from the limitation that the subject must have engaged in the behavior to answer the question. These results were surprising given that in clinical practice interest and pleasure are often disassociated. For example, one can be uninterested in dinner yet enjoy a meal, or interested in going to a party with friends and have an unrewarding experience. The term "interest" may be too inclusive or vague to have discriminating value. It may be confounded with other descriptors, for example anticipation, incentive, expectancy, motivation, appetite and satiation; interest might reflect an orienting response or a cognitive rehearsal of hoped to be pleasure. It would be important to determine, longitudinally, whether interest and pleasure co-vary with disease status, or whether one is disrupted before the other or more responsive to treatment. These findings may indicate that the neurophysiology of these related semantic constructs may be more similar than previously thought.

75. Efficacy of Two Years of Maintenance Treatment with Venlafaxine XR 75 mg/d to 225 mg/d in Patients with Recurrent Unipolar Major Depression

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Background: A recently completed 2.5-year recurrence prevention study demonstrated the long-term efficacy of venlafaxine extended-release (XR) in a dose range of 75 mg/d to 300 mg/d. However, in many countries, the approved maximum dose is 225 mg/d. Consequently, this analysis was done to determine the long-term probability of remaining well on 225 mg or less of venlafaxine XR in a population of patients with recurrent major depressive disorder (MDD).
Methods: In this multi-phase, multicenter, double-blind trial, outpatients with recurrent MDD (N=1096) were randomly assigned to 10 weeks of acute-phase treatment with venlafaxine XR (75 mg/d to 300 mg/d) or fluoxetine (20 mg/d to 60 mg/d) followed by a 6-month continuation phase and 2 consecutive 12-month maintenance phases for patients maintaining response/remission at the end of each phase. At the start of each maintenance period, venlafaxine XR responders were randomly assigned to receive double-blind treatment with venlafaxine XR or placebo, and fluoxetine responders continued to receive double-blind treatment with fluoxetine. For the purposes of this analysis, data were analyzed to assess the efficacy of venlafaxine XR in the subset of patients who achieved response/remission on 225 mg/d or less during the acute or continuation phases. The cumulative probability of these patients maintaining their response at 225mg/d or less in the subsequent maintenance phases, A and B, was the outcome of interest. Patients were considered to have had a loss of response if they had either a recurrence of depression or required a dose increase above 225 mg/d. Time to failure to maintain response was evaluated with Kaplan-Meier methods using log-rank tests for comparisons between venlafaxine XR and placebo.
Results: At maintenance phase A baseline, 144 patients were taking >225 mg/d of venlafaxine during the acute/continuation phase, and 114 patients were taking venlafaxine XR 225 mg/d or less. The latter group comprised the analysis population (venlafaxine XR: N=55; placebo: N=59). During the course of maintenance phases A and B, 7 patients in the venlafaxine XR group required a dose increase to 300 mg/d and 4 patients had a recurrence (4 overlapped on both); among patients assigned to placebo, 16 received a dose increase and 7 had a recurrence (7 overlapped on both). Kaplan-Meier probability estimates for maintaining response at 225 mg/d or less across the combined 24 months of maintenance phase treatment were 70% for venlafaxine XR and 38% for placebo (P=0.007).
Discussion: These data confirm the long-term efficacy of venlafaxine XR in maintaining response/remission among patients receiving doses in the range of 75 mg/d to 225 mg/d. The findings are consistent with the results of the full data set.

76. Development and Reliability of the SIGMA: A Structured Interview Guide for the Montgomery-Asberg Depression Rating Scale

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Sponsor: Robert L. Spitzer

Background: Observer-rated depression rating scales are used in clinical trials to enroll patients and to assess efficacy. The Montgomery-Asberg Depression Rating Scale (MADRS) was originally published without suggested probes for clinicians to use in gathering the information necessary to rate the items. Semi-structured interview guides for some rating scales have been found to improve inter-rater reliability. This report describes the development and test-retest reliability of a Structured Interview Guide for the MADRS (SIGMA). We also compared administration of the SIGMA in face-to-face interviews, over the telephone, and via videoconference.
Methods: Prior to the study, raters underwent reliability training consisting of a review of the scale's scoring conventions, and at least three practice interviews observed by a trainer. One hundred sixty-two test-retest interviews were then conducted by 81 rater pairs (six different raters). Each patient was interviewed twice, once by each rater. Interviews were conducted independently, with raters blind to each other's results. Interviews were conducted face-to-face, by telephone, or by videoconference, in order to examine the equivalence of these three methods.
Results: The intraclass correlation (ICC) for total scale score between raters was 0.93, p<0001. The mean score difference between SIGMA interviews conducted by the first and second interviewer was less than one point (20.49 vs 20.65), p = .717. All ten of the scale items had good (.70 to .80) to excellent (>.80) inter-rater reliability, with more than half of the items in the excellent range. Mean interview length was 25.78 minutes. The ICC for SIGMAs conducted face-to-face (N=30) was 0.93; 0.96 for pairs in which one SIGMA was done face-to-face and the other was done by videoconference (N= 30); and 0.90 between one face-to-face and one telephone administration (N=21). There was no significant difference among these correlations.
Discussion: This report describes the development and testing of a structured interview guide for the MADRS. The most stringent test of inter-rater reliability was used: independent test-retest interviews. This method best approximates the interrater agreement that would be achieved in clinical trials in which patients are assessed by a different rater at each visit. Although the training was fairly rigorous, it is

replicable. All raters in this study were experienced mental health clinicians, although they had varying degrees of prior experience with the MADRS scale. The level of agreement that can be attained with raters who have less clinical experience remains to be seen, although it is likely that the structured interview guide facilitates the use of this scale by less-experienced raters. To our knowledge, this is the first assessment of the reliability of the MADRS in which all test-retest interviews were independent, and in which agreement at the item level is reported. In this study, agreement on the total MADRS score was in the excellent range, and the reliability of all ten of the items was good to excellent. This study has demonstrated that with the use of the SIGMA, a group of interchangeable raters can achieve high reliability of MADRS total and item scores in a range of patients with depression. The extent to which this improvement in inter-rater agreement translates into improved signal detection in trials using the MADRS, remains to be demonstrated. Results also support the equivalence of remote administration of the SIGMA by both telephone and videoconference, to face-to-face administration.

77. Double-Blind Comparison of Escitalopram and Duloxetine in the Acute Treatment of Major Depressive Disorder

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Background: Previous studies have demonstrated that the selective serotonin reuptake inhibitor escitalopram has comparable or greater efficacy, while showing better tolerability, than the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine XR. The present study evaluates the efficacy and safety of escitalopram 10-20 mg/day versus another SNRI, duloxetine 60 mg/day, in the acute treatment of patients with moderate to severe major depressive disorder (MDD). Dosing was consistent with information in the FDA-approved package insert of both drugs.
Methods: Outpatients (aged 18-80 years) with DSM-IV diagnosed MDD (Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥26) were randomized to 8 weeks of double-blind treatment with escitalopram 10-20 mg/day (dose fixed at 10 mg/day for the first 4 weeks with optional up-titration to 20 mg/day thereafter) or duloxetine 60 mg/day. The primary efficacy endpoint was change from baseline at week 8 in MADRS total score using the last observation carried forward (LOCF) approach.
Results: Significantly more patients discontinued during 8 weeks of treatment in the duloxetine group (N=41/133) than in the escitalopram group (N=18/137), 31% vs. 13% (p=0.001), respectively. Mean baseline MADRS total scores were 31.0 for the escitalopram group and 31.6 for the duloxetine group. At week 8, escitalopram treatment resulted in significantly greater improvement compared with duloxetine on the prospectively-defined primary efficacy endpoint of change from baseline in MADRS total score using the LOCF approach (LSMD -2.42 [95% CI: -4.73, -0.11]; p=0.040). Moreover, the proportion of patients responding to escitalopram treatment (50% improvement in MADRS total score) was significantly greater in the escitalopram group than in the duloxetine group, 68% versus 52% (p=0.011; LOCF), respectively. Remission (MADRS≤10) rates were 44% in the escitalopram group and 38% in the duloxetine group. Escitalopram was better tolerated; significantly fewer escitalopram-treated patients discontinued due to adverse events compared with duloxetine (2% versus 13%; p=0.001). Serious adverse events were reported in one escitalopram-treated patient (1%) and 5 duloxetine-treated (4%) patients during the 8 week treatment period.
Discussion: These findings suggest that escitalopram is better tolerated and more effective than duloxetine in the treatment of MDD. These results, along with those from comparative studies with venlafaxine XR confirm that escitalopram has equal or greater efficacy in the treatment of MDD compared to SNRIs.

78. Further Evidence for a Metabotropic Glutamate Group II Antagonist Mechanism for the Induction of Antidepressant-Like Effects in Rodents

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Sponsor: Phil Skolnick

Background: Recent data has suggested that metabotropic glutamate (mGlu) 2/3 receptor antagonists might function as antidepressants. These data rely on effects of two compounds in neurochemical and behavioral models predictive of antidepressant efficacy. Additional support for a mechanistic role for mGlu2/3 receptors in antidepressant-like effects is needed.
Methods: Effects of LY341495 [(2S)-2-amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid] and an inactive isomer were studied in wildtype mice and mice devoid of mGlu2, mGlu3, or mGlu8 receptors in the forced-swim test assay, predictive of antidepressant efficacy, to bolster experimental evidence linking mGlu2/3 receptors to antidepressant-like efficacy.
Results: LY341495 but not an inactive isomer decreased immobility in the forced-swim test in mice and rats and decreased marble burying. Behavioral effects of LY341495 were markedly diminished in mGlu2 and mGlu3 but not in mGlu8 receptor knockout mice. In contrast, generally comparable antidepressant-like effects of imipramine were induced in all three mouse types. The effects of LY341495 were attenuated by the AMPA receptor antagonist NBQX and augmented by the tricyclic antidepressant imipramine. Effects of LY341495 on marble burying were diminished in mGlu2 but not mGlu3 or mGlu8 receptor knockout mice.
Discussion: The pharmacological findings with an inactive enantiomer of LY341495 and in mice devoid of mGlu2 or mGlu3 receptors firmly establishes mGlu2/3 receptors as the mechanism of action of the mGlu group II antagonists in in vivo assays predicting antidepressant and anxioltyic effects. The data also confirm that the role of these receptors is to augment synaptic glutamate at postsynaptic AMPA receptors.

79. Genome-Wide Profiling of Amygdala, Hippocampus, Striatum and Frontal Cortex Gene Expression in Mice with a Targeted Deletion of the Serotonin Transporter

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Background: The serotonin transporter (SERT, 5-HTT) is the single most important molecule regulating serotonergic neurotransmission. Being the primary target for a number of psychopharmacological targets such as serotonin reuptake inhibitors, it has been extensively investigated in pharmacological and genetic analyses. Mice with a targeted deletion of the serotonin transporter gene (SERT KO, SKO) display a number of behavioral, neurochemical and brain morphological alterations, such as increased anxiety, presynaptic serotonin 1A receptor downregulation, attenuated response to 3,4-methylenedioxymethamphetamine, and dysfunctionally malformed whisker barrelfields in the somatosensory cortex. However, changes at the level of gene expression have not been systematically analyzed in SKO mice on a genome-wide level. The aim of the current investigation was to compare the transcriptome of SKO with wild-type (WT) mice in four brain regions, specifically, amygdala, hippocampus, striatum and frontal cortex, which were chosen because of their relevance to neuropsychiatric disease.
Methods: Tissue from the specific brain regions of five male SKO and five WT mice was harvested by punching cryoslices with a microcan-

nula. RNA was amplified with T7 polymerase, reverse-transcribed and hybridized to a 33K custom spotted oligonucleotide array representing the entire mouse transcriptome. We analyzed a total of five biological replicates (individual SKO RNA compared to pooled RNA from WT mice), each of which was hybridrized in quadruplicate (technical replicates). Raw data were LOWESS-normalized and background-subtracted and analyzed by fold-change, focusing initially on neurotransmitter system genes.
Results: Most strikingly, we observed a global upregulation of the GABA-A transporter 4 (Slc6a11) and glutamic acid decarboxylase 2 (Gad2), plus several glutamate receptors, specifically, AMPA1, AMPA3, and metabotropic receptor 5. Other regulated genes related to neurotransmission included brain-derived neurotrophic factor, CCK and CCK-B receptor (specifically downregulated in striatum), and alpha 2a adrenergic receptor and thyrotropin releasing-hormone, which were downregulated in hippocampus. The dopamine receptor 3 was specifically upregulated in the cortex. We are currently expanding the analysis of our data to include cluster and pathway analyses, which should give further insight into differentially regulated genes in specific brain regions.
Discussion: Although the results from our experiments require experimental validation such as pharmacological studies, they support the notion of a complex interaction between serotonin and gabaergic and glutamatergic neurotransmission. In addition, the regional specificity of some of the gene expression changes observed herein calls for more detailed studies in the future and will help our understanding of neurotransmitter interactions at the gene expression level, which could in turn result in novel possibilities for targeted pharmacological interventions in neuropsychiatric diseases.

80. Modulation of AMPA Glutamate Receptor Trafficking in Treatment of Bipolar Disorder: Involvement of Antimanic Effect in Animal Model

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Sponsor: Past Travel Awardee, sanofi-aventis, 2005

Background: In recent years, there has been a growing appreciation that intracellular signaling cascades play a role in the pathophysiology and treatment of severe mood disorders. An important problem to address is how changes in intracellular molecules bringing about complex behavioral changes? It is our contention that these signaling cascades undoubtedly converge to regulate synaptic plasticity, and thereby information processing, in critical circuits mediating the affective, cognitive, motoric and somatic, manifestations of mood disorders. In this context, it is now clear that modification of the levels of synaptic AMPA receptors, in particular by receptor subunit trafficking, insertion and internalization, is a critically important mechanism for regulating various forms of synaptic and behavioral plasticity. Notably, AMPA receptor trafficking is regulated by signaling cascades known to be targets of mood stabilizers. We therefore undertook a series of studies to determine if lithium and VPA's effects on signaling cascades converge to regulate surface and/or synaptic AMPA receptors.
Methods: Synaptic or membrane GluR1 and GluR2 and GluR1 phosphorylation were determined in vivo and in vitro after lithium- and valproate- chronic treatment. To further characterize the role of synaptic AMPA receptor insertion in mediating facets of affective-like behaviors, we have used a Tat-peptide strategy. The Tat peptide, derived from the HIV coat, has been found to facilitate the transfer of peptides across not only plasma membranes, but also across the BBB. We utilized this TAT-peptide as a tool to dissect out the involvement of AMPA receptor in manic-like behavior.
Results: Chronic treatment of rats with therapeutically relevant concentrations of lithium or VPA reduced hippocampal synaptosomal GluR1 and GluR2 levels. The reduction in synaptic GluR1 and GluR2 by lithium and VPA was due to a reduction of surface GluR1 and GluR2 distribution onto the neuronal membrane as demonstrated by three independent assays in cultured hippocampal neurons. In addition, these agents induced a decrease in GluR1 phosphorylation at a specific PKA site (GluR1-p845), which is known to facilitate AMPA receptor insertion and opening of the sodium channel. GluR1p845 phosphorylation was also attenuated in hippocampus from lithium-or VPA-treated animals in vivo. Furthermore, Sp-cAMP treatment reversed the attenuation of phosphorylation by lithium and VPA and also brought GluR1 and GluR2 back to the surface, suggesting that phosphorylation of GluR1p845 is involved in the mechanism of GluR1 and GluR2 surface attenuation. In striking contrast, antidepressants, psychostimulants, dopamine agonists, and sleep deprivation have also been shown to increase phosphorylation and/or synaptic levels of GluR1 receptors. Tat-p845, which mimics the effect of lithium and VPA, attenuated GluR1 phosphorylation at its PKA site and reduced surface and synaptic GluR1/2 in hippocampal neurons in vitro and in vivo. Intra-hippocampal infusion of TAT-p845 peptide, or AMPA specific inhibitor GYKI54226 was able to attenuate amphetamine-induced hyperactivity and conditioned place preference in the mania animal model, suggesting that phosphorylation of GluR1 at p845 in hippocampus is essential for development of mania.
Discussion: These studies suggest that GluR1/2 trafficking may confer antidepressant /antimanic profiles to antidepressant and antimanic agents and regulation of glutamatergically mediated synaptic plasticity and may play a role in the treatment of bipolar disorder, and raises the possibility that agents more directly affecting synaptic GluR1/GluR2 may represent novel therapies for this devastating illness.

81. Genetic Association Study of the Amiloride-Sensitive Cation Channel 2 (ACCN2) Gene and Anxiety Spectrum Disorders

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Ion channels are involved in a wide-range of CNS functions and have been implicated in several neuropsychiatric disorders. Several rodent studies suggest that the acid-sensing ion channel ASIC1 may play a role in fear conditioning, a model for human anxiety disorders. In this study, we examined, for the first time, the human analogue of ASIC1, the amiloride-sensitive cation channel 2 gene (ACCN2), for its association with genetic risk across a range of anxiety spectrum phenotypes.
Methods: Using multivariate structural equation modeling, we selected twin pairs scoring at the extremes of a latent genetic risk factor that underlies susceptibility to neuroticism, major depression, generalized anxiety disorder, panic disorder, agoraphobia, and social phobia, from a sample of 9270 adult subjects who have participated in the population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders. One member from each selected pair for whom DNA was available was entered into a 2-stage, case-control association study for the ACCN2 gene. In the resulting sample of 589 cases and 539 controls, a total of 7 SNPs that represented the major allelic variation across the ACCN2 locus were screened in stage 1, the positive results of which were tested for replication in stage 2.
Results: While several markers or haplotypic combinations met threshold significance criteria in stage 1, their association was not replicated in stage 2. Post-hoc analyses did not reveal association to any of the specific psychiatric phenotypes.
Discussion: While the ACCN2 gene may play a role in rodent fear conditioning, we could not detect association to human anxiety spectrum disorders.

82. Morpholino Antisense Oligonucleotide-Mediated Knockdown of Tryptophan Hydroxylase-2 in a Discrete Subregion of Rat Dorsal Raphe Nucleus

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Background: Dorsal raphe nucleus (DRN), a major source of serotonin in the forebrain, is composed of anatomically and functionally distinct subregions that are implicated in different anxiety and depressive disorders. In order to investigate the function of specific subregions in discrete tasks, we have explored the use of a new lissamine-tagged morpholino antisense oligonucleotide (MAO) to knockdown trypotophan hydroxylase-2 (TPH2), the rate-limiting enzyme for serotonin biosynthesis, selectively in the mid-DRN. This MAO strategy offers greater anatomical specificity than traditional lesion or pharmacological strategies. Furthermore, compared to traditional DNA antisense oligonucleotides, MAO-mediated knockdown has enhanced specificity, efficiency and stability.
Methods: The MAO spanned an exon-intron junction and was designed to prevent normal splicing so that a nonfunctional protein is formed. Adult male Sprague-Dawley rats were injected in the mid-rostral DRN with 0.2ml of saline, random sequence MAO or antisense TPH2 MAO; unoperated animals were also used as an additional control group. After three days of recovery, rats were sacrificed for microscopic examination of lissamine signal for cellular uptake and western blot or immunohistochemistry to examine expression of TPH protein levels, neuronal damage, anatomical and target specificity.
Results: Lissamine labeled MAO were efficiently transported into neuronal cytoplasm and nuclei after injection; these neurons had normal morphology. Antisense TPH2 MAO reduced expression of TPH protein in the mid-rostral DRN while protein levels in the caudal DRN were preserved. This was confirmed by both western blot and immunohistochemistry. Control MAO did not alter TPH protein levels as compared to vehicle controls or unoperated animals. MAO did not induce obvious tissue damage and did not induce gliosis or apoptosis. Interestingly, knockdown of TPH appeared to also knockdown Pet-1 protein levels, a serotonin-selective transcription factor. However, serotonin transporter protein levels were normal at the site of MAO injection and the serotonergic neurons appeared healthy. Finally, preliminary data indicates that MAO-mediated knockdown of TPH2 in mid-rostral DRN may increase anxiety-like behavior in the open field test without altering general locomotor activity.
Discussion: These results illustrate MAO-mediated knockdown as promising new tool to study and manipulate gene function. Furthermore, the anatomical specificity of MAO will allow us to investigate the role of TPH in specific subregions of DRN on behavior and physiology.

83. Pharmacological Profile of the 5-HT_{2C} Receptor Agonist WAY-163909: Therapeutic Potential in Multiple Indications

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Sponsor: Jack Bergman

Background: The 5-HT_{2C} receptor subtype has been implicated in a wide variety of conditions including obesity, anxiety, depression, obsessive compulsive disorder, and schizophrenia, and as a consequence has received considerable attention as a target for drug discovery. Here we review the pharmacological profile of WAY-163909 ((7bR,10aR)-1,2,3,4,8,9,10,10a-octahydro-7bH-cyclopenta[b][1,4] diazepino[6,7,1hi]indole), a novel 5-HT_{2C} receptor selective agonist.
Methods: WAY-163909 was evaluated for its effects on food intake (normal and obese rats and mice following acute and chronic administration) and in models predictive of antipsychotic (antagonism of apomorphine-induced behaviors, conditioned avoidance responding (CAR)) and antidepressant (forced swim test, resident-intruder, learned helplessness and olfactory bulbectomy models) efficacy, and a model of obsessive-compulsive disorder (schedule-induced polydipsia). These data were further amplified by neurochemical and electrophysiological studies.
Results: Acutely, WAY-163909 produced dose-dependent decreases in food intake in normal Sprague-Dawley rats, obese Zucker rats and diet-induced obese mice (MED 3 mg/kg ip), effects that were maintained after 10-days chronic administration. In the rat CAR model, WAY-163909 reduced avoidance responses while increasing escapes (MEDs = 1.0 mg/kg and 3.0 mg/kg following ip and po dosing, respectively) and blocked apomorphine-induced climbing (MED = 5.4 mg/kg ip) without substantially affecting stereotypy up to 30 mg/kg. In this dose range, WAY-163909 did not produce significant levels of catalepsy in mice. Acute and chronic (21 day) treatment with WAY-163909 (1-10 mg/kg ip) produced dose-dependent decreases in the number of spontaneously-active A10, but not A9, dopamine neurons, and a dose of 10 mg/kg sc produced a selective decrease in nucleus accumbens dopamine (relative to striatum)and a modest increase in prefrontal cortex. WAY-163909 significantly decreased immobility with an MED of 10 mg/kg ip in the forced swim test in both SD and WKY rats, and selectively reduced the aggressive behavior of resident rats during social encounters at doses that do not result in motor impairment (MED $\leqq$ 0.3 mg/kg, sc). Administration of WAY-163909 for five days at 30 mg/kg ip produced dose-dependent decreases in both escape latency and escape failures in the learned helplessness model. Furthermore, WAY-163909 (3 mg/kg, ip administered for either 5 or 21 days) decreased the hyperactivity associated with olfactory bulbectomy without affecting levels of activity in sham-operated rats following both short-term and long-term treatment, consistent with rapid onset antidepressant-like effects. Finally, WAY-163909 also decreased adjunctive drinking in the schedule-induced polydipsia model with an MED of 3 mg/kg.
Discussion: Consistent with a potential therapeutic utility in obesity, schizophrenia and depression, WAY-163909 was found to have robust dose-dependent effects in animal models of feeding, antipsychotic-like and antidepressant-like activity.

84. The COMT 158val/val Genotype is Associated with Lower Probability of Antidepressant Treatment Response

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Sponsor: Thomas Schlaepfer

Background: Affective disorders are characterized by a life-time prevalence of up to 17% and a strong genetic background (1). Preclinical studies support the notion of abnormal catecholamine transmission in the pathogenesis of affective disorders, possibly conferred by a dysfunction of the enzyme catechol-O-methyltransferase (COMT), which is crucial in monoamine degradation. Increased COMT activity in erythrocytes was associated with major depression, particularly in male patients (2). The functional COMT val158met polymorphism has repeatedly been investigated for association in affective disorders, however with inconsistent results. Pharmacogenetic studies recently suggested the COMT 158met allele to be negatively correlated with response to antidepressant drug treatment (3, 4). In this present study, a large sample of patients with affective disorders was investigated in an attempt to further clarify the influence of COMT gene variation on treatment response in affective disorders.
Methods: A sample of 295 Caucasian patients with affective disorders according to DSM-IV (major depression: n=234, bipolar disorder: n=61) was characterized for family history of psychiatric disorders and genotyped for the COMT val158met variant according to published protocols (5). Weekly HAMD scores during antidepressant treatment with SSRIs, NSRIs or mirtazapine were assessed. Statistical

analysis in the overall sample and subgroups stratified for diagnosis, gender and family history of psychiatric diseases was performed using multivariate ANOVA (dependent variable: change of HAMD score between single weeks and admission as compared to changes in HAMD scores between week one and admission; independent variable: COMT val158met genotype; adjusted for the remaining variables) with Bonferroni post hoc test.
Results: In the whole sample of patients with affective disorders, the COMT 158val/val genotype conferred a significant risk of non-response after six weeks of treatment (p=0.01). Stratification into disorder groups revealed this effect to be particularly strong in patients with major depression (p=0.001), male patients (p=0.009) and patients with a positive history of psychiatric disorders in first-degree relatives (p=0.010).
Discussion: Our results suggest a negative effect of the higher activity COMT 158val/val genotype on antidepressant treatment response in patients with major depression. This finding, although in contrast to previous studies (3, 4), is in accordance with observations of elevated erythrocyte COMT activity in depression, particularly in male patients (2). Further support originates from pharmacological studies showing that the COMT inhibitor tolcapone reverses an anhedonic state in a rat model of depression and reduces symptom severity in the treatment of major depression (6, 7). In conclusion, in patients homozygous for the higher activity COMT 158val allele, the consecutively decreased availability of the monoamines norepinephrine and dopamine might impair the efficacy of antidepressants during the first six weeks of pharmacological treatment in major depression.
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85. Is Generalized Anxiety Disorder an Internalizing Distress Disorder?

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Sponsor: Past Travel Awardee, ECNP-ACNP Fellow, 2004

Background: Factor models propose a hierarchical factor structure of psychopathology with two correlated higher order dimensions - externalizing and internalizing. The latter further splits on a lower hierarchical level into an anxious-misery/distress and a fear subfactor. It has been suggested that Generalized Anxiety Disorder (GAD) is more closely related to major depression and dysthymia (internalizing distress dimension) than to phobias and panic disorder (internalizing fear dimension). Such factor models that are based on a limited number of cross-sectional diagnoses from adult samples have been used to argue for focusing etiological research and diagnostic classification on these hierarchical structures rather than on their varied manifestations as separate disorders. However, only disorders with the same risk factors should be lumped into one category. Aim of this paper is therefore to test in a community sample of youth whether GAD has the same risk factors as the other internalizing-distress disorders.
Methods: Data come from a representative community sample of 3,021 adolescents and young adults aged 14 – 24 at baseline and prospectively followed-up over a period of 10 years. Mental disorders were assessed using the Munich Composite International Diagnostic Interview (M-CIDI) and DSM-IV criteria. Odds Ratios (OR) and respective 95%-Confidence Intervals were estimated by logistic regression analyses adjusting for age and gender using several potential baseline risk factors as independent variables and the incidence of internalizing-fear disorders (social phobia, specific phobia, panic disorder, agoraphobia; N = 198), GAD (including subthreshold cases; N = 66), internalizing distress disorders (major depression, dysthymia; N = 205), and externalizing disorders (abuse and dependence of alcohol and illicit substances, conduct disorder/antisocial personality disorder; N = 195) as the dependent variables.
Results: The cumulated age of onset distribution reveals that 50% of cases with internalizing fear disorders, externalizing disorders, GAD, and internalizing distress disorders have emerged up to age 9, 15, 18, and 18 respectively. With some deviances in younger age, GAD shows a similar age of onset slope as internalizing distress disorders. All of the distal (parental anxiety disorder, parental depressive disorder, parental substance use disorder, behavioural inhibition, early adversity) and proximal risk variables (trauma at age 10 or later, overall burden due to life events and life conditions, daily hassles, lower levels of self-worth and competencies as well as coping) were associated with the incidence of one or more of the diagnostic categories (internalizing fear disorder, GAD, internalizing distress disorder, and/or externalizing disorders). Risk factors for incident GAD were parental anxiety disorder (OR: 2.0), parental substance use disorder (OR: 1.7), behavioural inhibition (OR: 1.4), daily hassles (OR: 1.3), and low coping expectations (OR: 1.4). Burden due to life events/conditions was additionally relevant among subjects with parental anxiety disorder (OR: 1.4) or parental substance use disorder (OR: 1.4). This risk pattern differed from the ones of the other internalizing distress disorders, the internalizing fear disorders, and the externalizing disorders.
Discussion: Whereas the age of onset pattern of GAD suggests a relation with internalizing distress disorders rather than internalizing fear disorders, the risk factors for GAD neither do completely correspond with the ones found for internalizing distress disorders nor with the ones found for internalizing fear disorders or externalizing disorders. These findings argue for caution regarding lumping disorders together in broader categories, especially if the goal is to reveal true psychopathological processes.

86. Effects of Modulation of Group Ii mGluRs on Conditioned Fear Stress-Induced Neurochemical and Behavioral Responses

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Sponsor: Karoly Mirnics

Background: Excitatory neurotransmitter glutamate (Glu) is implicated in the pathophysiological mechanisms of anxiety. Drugs targeting group II metabotropic glutamate receptors (mGluR2 and mGluR3) show efficacy in animal models of anxiety such as fear conditioning. Group II metabotropic glutamate receptors are localized at the presynaptic terminals of glutamatergic synapses in the rodent and primate amygdala, the structure which neuronal circuits play a crucial role in the development of conditioned fear and anxiety. We examined the effect of conditioned fear stress on extracellular Glu concentrations in basolateral amygdala (BLA) and its regulation by Group II mGluRs to clarify the role of glutamatergic input into the amygdala in the pathophysiology of fear and anxiety.
Methods: Glu efflux in the BLA in animals subjected to conditioned stress (CFS) paradigm was measured by in vivo microdialysis coupled with HPLC-ECD. In the CFS paradigm animals were subjected to the sequence of 10 combinations of light followed by a foot-shock (FS)(1.5 mA, 0.5 s duration) during the 10 min period. 120 min later rats were presented with a flashing light, and freezing behavior was observed and scored.
Results: Exposure of animals to CFS paradigm resulted in a significant increase of Glu (180%) in microdialysates collected from BLA. mGluR2/3 agonist, LY379268 (1 and 10 mg/kg s.c.) administered prior to FS, attenuated CFS-induced increase in Glu concentrations in microdialysates collected from BLA. LY379268 administered in BLA through the reverse microdialysis (0.1- 10 μM) dose-dependently reduced Glu concentrations in microdialysates from BLA without the effect on the basal concentrations of Glu. Administration of

LY379268 also attenuated expression of freezing behavior in animals in response to presentation of CS. Administration of mGluR2 selective positive modulators LY487379 (100 mg/kg, i.p.) and LY566332 (30 mg/kg s.c) produced similar results, attenuating stress-induced Glu release in the BLA.
Discussion: Our findings demonstrate that modulation of Group II receptors by a mGluR2/3 agonist or mGluR2 selective positive modulators attenuates CFS-induced activation of glutamatergic system in amygdala, suggesting that this mechanism may be beneficial for anxiolytic properties of these compounds.

87. 5-HT_6 Receptor Stimulation Regulates BDNF and Induces Neuronal Plasticity: Potential Mechanisms for Antidepressant Drug Action and Neuroprotection

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Background: Significant advances in our understanding of the 5-HT_6 receptor have been made through the recent development of the 5-HT_6 agonists, WAY-208466 and WAY-181187. Both agents are high affinity selective full agonists that can increase extracellular levels of GABA in prefrontal cortex, hippocampus and striatum and decrease stimulated levels of glutamate induced by KCl or sodium azide as evaluated in a hippocampal slice preparation. Herein we extend our previous results and report that 5-HT_6 agonists increase the neurotrophin BDNF and possess antidepressant/anxiolytic-like activity and efficacy in a model of permanent focal ischemia.
Methods: Primary cortical neuron cultures were prepared from E16 rat embryos and incubated overnight. Cultures were treated with 10 uM WAY-208466 or WAY-181187 over a 5 day period. The specific time-point at which BDNF protein levels were maximal was subsequently used as the time point to evaluate concentration dependent effects. The amount of BDNF protein was quantified by a BDNF sandwich ELISA using 50 ug of protein (BDNF Emax ImmunoAssay System, Promega). Schedule-induced polydipsia was performed in rats on a fixed-time 60 sec food schedule and water was freely available. The amount of drinking during a 2 hour test session was measured. The Forced Swim Test was performed as described by Detke et al., Psychopharmacology, 1995, 121:66-72. The pMCAO (permanent Middle Cerebral Artery Occlusion) model was accomplished through cauterization of the MCA with subsequent testing of sensorimotor function every 3 days during a 3 -week interval post–ischemia.
Results: Previously we reported that 5-HT_6 agonist activity could increase neurite outgrowth and survival in primary cultures. In an attempt to help define mechanisms for this effect we evaluated the ability of these compounds to induce an increase in BDNF protein levels in primary cortical and hippocampal neurons. WAY-208466 and WAY-181187 increased BDNF protein levels in a concentration- and time-dependent manner with peak effects occurring at the 24 hr time point. In parallel studies and based upon the ability of the compounds to induce effects on BDNF which has been implicated in antidepressant activity as well as neuronal plasticity behavioral evaluations were performed for 5-HT_6 agonists in models of depression and anxiety as well as neuroprotection. In Schedule-induced Polydipsia which is a model of obsessive-compulsive disorder WAY-208466 and WAY-181187 decrease adjunctive drinking without affecting normal water or food intake. Recent data evaluating WAY-208466 in the Forced Swim Test demonstrates antidepressant-like activity of a 5-HT_6 agonist as the compound induced a decrease in immobility time but did not have significant effects on either swimming or climbing behavior. Furthermore in a pMCAO model, WAY-181187 administered 4 hrs following the ischemic insult significantly reverses neurological deficits as observed over a 21-day period.
Discussion: Taken together, 5-HT_6 receptor agonists (WAY-208466 and WAY-181187) increase BDNF protein levels in a time and concentration-dependent manner, demonstrate antidepressant-like and anxiolytic activity, and neuroprotective properties both in vitro and in vivo. This would suggest among the multiple 5-HT receptors that would be stimulated by antidepressants that block serotonergic reuptake that the 5-HT_6 receptor may play an important role and appears at least partly to involve BDNF as a mechanism. The combined effects denoted for a 5-HT_6 agonist have multiple implications for drug development strategies associated with indications that have co-morbid mood disorder and neurodegenerative aspects.

88. Asenapine Reverses Chronic Mild Stress-Induced Anhedonia in the Absence of an Hedonic Profile

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Background: Chronic mild stress (CMS) can be used to induce an anhedonic state in rats via hypoactivity in the mesolimbic dopamine pathway; the effect is reversible by chronic antidepressant treatment. Pharmacological stimulation of the mesolimbic pathway can be assessed using an intracranial self-stimulation (ICSS) protocol. Asenapine is a novel psychopharmacologic agent being developed for the treatment of schizophrenia and bipolar disorder. We explored the effects of asenapine in both CMS and ICSS.
Methods: In the CMS protocol, 2 months before drug testing, male Wistar rats were trained to consume a 1% sucrose solution over 8 one-hour baseline tests. On the basis of these tests the animals were divided into control and stress groups, the latter being subjected to the CMS procedure for 7 consecutive weeks. The groups were further divided into drug and vehicle groups and given asenapine 0.06, 0.2, or 0.6 mg/kg intraperitoneally (i.p.) twice daily; imipramine 10 mg/kg (i.p.) once daily; or vehicle. In the ICSS protocol, a bipolar electrode was implanted into the ventral tegmental area of male Sprague-Dawley rats. During sessions each lever press delivered a biphasic square wave stimulus train (150 ms, pulse width 500 μs, delay 100 μs) to the electrode; after initial lever training, a variable interval 3-second schedule was used during extinction/reacquisition and rate-frequency training. After stability was achieved, drug testing began. An ascending rate-frequency protocol with 11 conditions lasting 2 minutes each was used, with the frequency increasing from 10 to 175 Hz. From the rate-frequency curves, the locus of rise (LOR) and maximal rate of responding (MAX) were calculated. Asenapine 0.01, 0.03, 0.1, or 0.3 mg/kg subcutaneously (s.c.) or vehicle was administered 30 minutes before testing. As a positive control, a dose of cocaine (5.0 mg/kg, s.c.) was included.
Results: CMS caused an initial 34% decrease in sucrose consumption ($P<0.001$). Imipramine and asenapine were inactive in the control groups. In stressed animals, imipramine was effective ($P<0.001$), with sucrose consumption indistinguishable from that of controls by week 4 ($P<0.05$). Asenapine was similarly effective ($P<0.001$). Significant treatment effects were seen at the 2 higher doses of asenapine (0.2 mg/kg, $P=0.021$; 0.6 mg/kg, $P<0.001$), with the highest dose reaching control values by week 3 ($P<0.05$). In the ICSS protocol, asenapine 0.1 and 0.3 mg/kg significantly increased LOR (by 5% and 9% from baseline, respectively), whereas asenapine 0.3 mg/kg significantly decreased MAX (by 59% from baseline) ($P<0.05$). In contrast, cocaine 5.0 mg/kg decreased LOR and left MAX unchanged.
Discussion: We used 2 routes of administration for operational reasons. Pharmacokinetic analysis shows that drug exposure from asenapine 0.6 mg/kg i.p. is equivalent to 0.3 mg/kg s.c., confirming that the 2 studies bear comparison. The ICSS data indicate that asenapine, given acutely, does not have an overtly hedonic profile. The equivalent dose administered on a subchronic basis to CMS animals was as

effective as imipramine in decreasing stress-induced anhedonia, and this amelioration was not due to an acute stimulation. These findings suggest that asenapine may have therapeutic utility in bipolar and other affective disorders.

89. Aggression Is Associated with Altered Expression of Genes for Neurotransmission and Neuron Structure in the Human Prefrontal Cortex

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Sponsor: Travel Awardee, sanofi-aventis, 2006

Background: Aggressive behaviors are associated with several psychiatric disorders such as psychosis or substance use disorders and can be a consequence of brain injury. Potential causal factors for aggressive behaviors include genes and childhood adversity. Aggression is also associated with neurotransmitter alterations, but the responsible genes are unknown. We sought to identify candidate genes and further elucidate the pathophysiology by determining gene expression alterations associated with aggression in postmortem human prefrontal cortex.
Methods: Using Affymetrix U133A arrays, postmortem samples from dorsolateral (area 9) and orbital prefrontal cortex (area 47) were examined from 36 cases (27 males, 9 females; 19 normal non-psychiatric, 17 suicide with or without Major Depression) ranging from 13 to 73 years of age (43±20 years, mean±SD). All subjects had negative toxicological screens and had a psychological autopsy to determine Axis I and II diagnoses. The Brown-Goodwin scale measured lifetime aggression with scores divided into verbal (verbal assault and irritability) and physical aggression (direct and indirect assault). Correlations between gene expression and aggression scores were assessed using the Spearman correlation. Genes with p-values < 0.01 and fold-change threshold of 1.2 between the most aggressive and least aggressive groups were considered significantly different.
Results: Lifetime aggression scores ranged from 10 to 27 and declined with age (R2=0.09; p=0.06). Physical aggression, but not verbal aggression, was greater in males than females (physical: z=-3.9, p=0.0001; verbal: z=1.31, p=0.19). We found more gene alterations associated with physical than verbal aggression, and more in the orbital than in the dorsolateral prefrontal cortex [physical aggression: 114 genes in A9 vs. 470 genes in A47; verbal aggression: 18 genes in A9 vs. 151 in A47]. Gene expression alterations were also more pronounced in area 47 than area 9 (higher fold change). Changes associated with physical aggression related mainly to GABA/Glutamate system synaptic transmission (GABRA5, 179%; GAD1, 165%; SLCA1, 154%; GRM1, 136%; GRM5, 129%; AMPA3, 134%), and also involved the serotonin system (HT5A receptor, 130%). The changes affected neurotransmission through receptors and channels (CB1, 154%, CACNA2D1, 157%, Synapsin SYNII, 165%), trophic factors (NTRK2, 153%) and growth factors (GMFbeta, 195%; FGF2, FGFR1, 125%; IGF1R, 130%), as well as through genes implicated in cell adhesion and neuron migration (NRXN1, 169%; NCAM, 155%). Changes associated with verbal aggression related mostly to inflammatory and stress responses (SH3GLB1, 128%; SERP1, 121%; TDO2, 69%; IGF1R, 130%). Changes were confirmed for some of the selected genes at the expression level using quantitative Real-time PCR (GABRA5, 120%; GMFbeta, 201%; NRXN1, 135%).
Discussion: The gene expression changes associated with aggression suggest an alteration in the relative balance of excitatory and inhibitory neurotransmission in the prefrontal cortex. Further study of these genes may demonstrate genes with a causal role in aggressive behaviors and indicate new treatment targets for pathological aggression.

90. Interactions of SERT & BDNF: A Complex Genetic Model of Depression

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Sponsor: Gian C. Salmoiraghi

Background: BDNF and SERT, both of which have been associated with psychopathological states, are important genes in brain development and in functions related to memory and emotion. Genetic variations of the BDNF (val66met) and SERT gene (5-HTTLPR) affect the function of these proteins in neurons and predict variation in human memory and in fear behavior. Our previous work has shown that the S allele of 5-HTTLPR affects the integrity, function and connectivity, and presumably development of a neural circuit linking amygdala and rostral anterior cingulate circuitry (Pezawas et al., 2005), a circuitry related to anxious temperament and depression in the presence of environmental adversity. Additionally, we could show that val66met BDNF affects the development and function of brain circuitries (hippocampus, DLPFC) prominently implicated in aspects cognitive functioning (e.g. working memory) (Egan et al 2003, Pezawas et al. 2004). Convergent evidence links BDNF to depression, such as data showing association of the functional val66met BDNF polymorphism with increased risk for mood disorders, for temperamental traits related to mood disorders, and associated increases of BDNF expression after electroconvulsive therapy and antidepressive SSRI treatment. These data implicating a biological interaction of BDNF with 5-HTTLPR-dependent signaling suggest a molecular mechanism that could support an epistatic interaction between the functional variants in these genes in risk for depression. This possibility has been explored to a limited degree in animals genetically engineered to be hypomorphic at both genes. We hypothesized that the "insufficient" met BDNF allele does not translate the S allele effect of 5-HTTLPR and therefore protects the subject from significant changes in subgenual cingulate and amygdala volume, which is reflected in functional connectivity data of this brain circuitry.
Methods: We investigated high-resolution anatomical magnetic resonance images (MRI) of 111 normal healthy volunteers (Caucasians of European ancestry) without any psychiatric life-time history using optimized voxel-based morphometry (VBM), a sophisticated fully automated morphological imaging technique, which allows a statistical comparison of gray matter volume on a voxel-by-voxel basis. Furthermore, structural connectivity data were analyzed using SPM2.
Results: Consistent with our initial hypothesis, we found a significant increased 5-HTTLPR S allele volume loss of the subgenual cingulate and amygdala ($p<0.001$) in val/val BDNF carriers compared to met BDNF genotype. Structural connectivity and behavioral data reflected this relationship ($p<0.001$).
Discussion: The met BDNF allele may be a protective genetic factor for depression, because it only insufficiently translates 5-HTTLPR S allele dependent structural and functional changes, which are related to depression. *References: Pezawas, L. et al. J. Neurosci. 24, 10099-10102 (2004). Pezawas, L. et al. Nat Neurosci 8, 828-34 (2005). Hariri, A. R. et al. Science 297, 400-3 (2002). Hariri, A. R. et al. J Neurosci 23, 6690-4 (2003). Egan, M. F. et al. Cell 112, 257-69 (2003).*

91. Hippocampal Neurogenesis is Required for Lasting Behavioral Effects of Fluoxetine on the Rodent Forced Swim Test

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Neurogenesis in the dentate gyrus of the hippocampal formation has been causally linked to the anxiolytic effects of antidepressant drugs in animals, but it is unclear whether this relationship

extends to depression models. Furthermore, it is not known if increasing neurogenesis, a potentially lasting structural change, mediates lasting antidepresssant behavioral effects in the drug-free state.
Methods: Here we probed the dependence of the neurobehavioral effects of fluoxetine on hippocampal neurogenesis using animal models of learned helplessness and depression. We primarily utilized the forced swim test (FST), a widely used animal test predictive of antidepressant responses and depressed-like states, as a behavioral endpoint.
Results: One week of daily exposure to the antidepressant fluoxetine (20mg/kg) gave rise to decreased FST immobility when assayed 1 month after the last fluoxetine dose (control immobility = 60% +/- 3.8, fluoxetine immobility = 38.6% +/- 3.3, $p<0.01$). This behavioral effect of fluoxetine was absent when hippocampal neurogenesis was ablated by midcranial irradiation (immobility = 57.2% +/- 5.3, $p< 0.5$ relative to control). Irradiation alone had no effect on the FST relative to control (immobility = 63.3% +/- 6.0, n.s.). To determine whether one week of fluoxetine exposure was sufficient to upregulate hippocampal neurogenesis, we injected animals with the mitotic label BrdU during the week of treatment. Fluoxetine treatment indeed increased the number of BrdU+ neurons observed one month later (control = 1269 +/- 108, fluoxetine = 2143+/- 277, $p<0.05$). The number of Doublecortin-positive immature neurons was unaffected by fluoxetine ($p=0.23$), illustrating the transient effect on neurogenesis by the brief fluoxetine exposure 1 month earlier and indicating that prolonged neurogenesis upregulation is not required for the antidepressant effect of fluoxetine. We are now examining the potential lasting behavioral effects of transiently upregulating neurogenesis using the chronic mild stress animal model of depression.
Discussion: Taken together, these results demonstrate that brief fluoxetine exposure exerts long-lasting behavioral effects, relevant to depression-like behavior in rodents, through a neurogenesis-dependent mechanism.

92. Potential Involvement of the Dopamine Receptor D1 in Obsessive-Compulsive Disorder

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Sponsor: James L Kennedy

Background: Obsessive-Compulsive Disorder (OCD) occurs in 1 to 3 per cent of the population and is among the 10 leading causes of disability worldwide. Genetic factors are believed to play a major role in the etiology of OCD. Disturbances of the dopaminergic pathways have been implicated in the disorder based on the utility of dopamine blocking agents in OCD, and animal models, including induction of compulsive lever-pressing in rats specifically with D1 antagonism (Joel & Doljansky, 2003).
Methods: We investigated possible genetic associations between polymorphisms in DRD1 and OCD within a total of 404 nuclear families collected independently from three North American centres (Toronto, Ann Arbor, Buffalo). Analysis was performed using the Family Based Association Test (FBAT) on 5 SNPs in DRD1: -1251, rs686 (BspI), rs4532 (DdeI), rs265981 (HaeIII) and rs5326.
Results: The results of this analysis demonstrated a nominally significant association with the rs5326 polymorphism ($p=0.047$). The remaining SNPs did not reveal an association with OCD. The global haplotype analysis did not show significant association between DRD1 and susceptibility to OCD. However, a gender based analysis demonstrated a significant correlation between males and the -1251 polymorphism ($p=0.034$).
Discussion: Our results suggest that sequence variation in DRD1 may be associated with susceptibility to OCD, particularly in males. Further study of this gene for its association based on larger samples is warranted.

93. Antidepressant-Like Effect in the Swimming Test and Increased Cell Proliferation/Survival in Hippocampus of Adult C3H/HeN Mice Chronically Treated with the Melatonin (MLT) Receptor Ligand Luzindole

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Background: Depression remains the most prevalent mental disorder in the United States, and is the leading cause of disability affecting an estimated 10 million adults (Murray et al., World Health Organization, 1996; Am Psychiatr Assoc, DSM-IV, 1994). Antidepressants targeting neuronal transporters and G-protein coupled receptors for serotonin and norepinephrine remain the mainstay of treatment strategies. Antidepressants with novel mechanisms including actions at MT_1 and MT_2 MLT receptors are under development (Roth et al., Nat Rev Drug Discov 3:353,2004; Dubocovich, COID 7: 670,2006). Chronic antidepressant treatment with various chemical classes including the SSRI, fluoxetine, has been shown to increase cell proliferation and neuronal cell survival in the subgranular zone (SGZ) of the dentate gyrus (DG) in the hippocampus (Santarelli et al., 2003). We previously reported that acute treatment with luzindole, a competitive MLT receptor antagonist, shows antidepressant-like effects in an animal model of learned helplessness (Dubocovich et al., Eur J Pharmacol 182:313,1990) through actions on the MT_2 MLT receptor (Sumaya et al., J Pineal Res. 39:170, 2005). The goal of this study was to assess the effects of chronic treatment with luzindole or fluoxetine on cell proliferation and survival in the SGZ of the hippocampus and to measure the behavioral effects in the forced swimming test in C3H/HeN mice.
Methods: Male C3H/HeN mice (2-3-months-old) kept in a 14:10 light/dark cycle were treated ad libitum with vehicle, luzindole (0.02 mg/ml) or fluoxetine (0.08 mg/ml) added to drinking water for 21 days. At day 20 mice were subjected to a 6 min swimming test to assess time of immobility in 4 sec (Sumaya et al., 2005). At day 21 mice were injected with BrdU every 2 hours (75mg/kg x 4,ip) and sacrificed either 1 day (cell proliferation) or 27 days (cell survival and phenotype) after drug withdrawal. The brains were dissected and hippocampal sections (30 mm) were stained using the hydrogen peroxide method.
Results: Swimming test results revealed significant decreases in depressive-like symptoms (immobility) in groups treated with luzindole(148±7.1 sec, n=20, $p<0.05$) or fluoxetine (68±11, n=10, $p<0.001$) as compared to vehicle (178±7.6 sec, n=20). The more robust decrease in immobility was observed with fluoxetine. Luzindole and fluoxetine significantly increased the total number of dividing, BrdU positive cells (43±4.7 cells, n=9, $p<0.05$; 63±4.9 cells, n= 8, $p< 0.001$, respectively) in the SGZ across 12 hippocampal sections when compared with vehicle treated (25.6±2.5 cells, n= 8, $p<0.0001$). The number of survival cell, 27 days after the last BrdU injection was still significantly higher in SGZ from luzindole treated mice than in those treated with vehicle (n=10, $p< 0.001$). Results from double fluorescence neuronal (NeuN (neuron)/BrdU) or glial (GFAP (astroglia)/BrdU) staining demonstrated preferential neuronal survival.
Discussion: This is the first report demonstrating that the MLT ligand luzindole, like fluoxetine, shows antidepressant-like effects with chronic treatment in the C3H/HeN mouse and increased cell proliferation and survival in the SGZ of the hippocampus. These results add to the large number of drugs with antidepressant properties shown to promote neurogenesis in the mammalian brain (Duman et al., Biol Psych 56:140,2004). The receptor mechanisms (MT_1 and/or MT_2) by which luzindole increases cell proliferation and survival is under investigation. *Supported by MH 42922 to MLD.*

94. Effects of Adding Risperidone Long-Acting Injectable to Treatment-As-Usual in Patients with Frequently Relapsing Bipolar Disorder

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Background: An important subset of patients with bipolar disorder (BD) relapses frequently and experiences particularly high levels of morbidity and poor outcomes. Currently available maintenance treatments can be problematic due to incomplete efficacy and safety and tolerability issues. Patient noncompliance with existing therapies due to these limitations is associated with repeated relapse and increased clinical instability. This trial evaluates the effect of the addition of risperidone long-acting injectable (RLAI) to treatment-as-usual (TAU) on mood symptom control and functioning in patients with frequently relapsing BD (FRBD).
Methods: Patients with FRBD (defined for this study as those experiencing ≥4 episodes requiring clinical intervention in the past 12 months [including ≥2 episodes in the past 6 months]) received open-label (OL) augmentation of TAU with RLAI (25-50 mg) for 16 weeks. Remitters (defined as Young Mania Rating Scale [YMRS] ≤10 and Montgomery-Åsberg Depression Rating Scale [MADRS] ≤10 over the last 4 weeks of the OL phase) were eligible for randomization to placebo or RLAI in a double-blind (DB), 52-week, relapse-prevention study. Measures included MADRS, YMRS, and the Clinical Global Impressions–Severity (CGI-S) scale. OL stabilization phase results for 275 patients are reported.
Results: At baseline, 58% of patients were moderately to severely ill by CGI-S, 37% scored ≥20 on YMRS, and 38% scored ≥20 on MADRS. Mean (±SD) YMRS and MADRS scores were 13.5±11.7 and 11.0±10.5, respectively. Seventy-seven percent of patients completed the OL phase; 49% met remission criteria and were eligible to enter the DB phase; 25.5% did not meet remission criteria but continued OL RLAI treatment. Reasons for discontinuation from the OL phase included: adverse events (5.5%); lost to follow-up (5.5%); noncompliance (<1%); protocol violation (1%); and withdrawal of consent (8%). At OL endpoint, the percentage of patients with CGI-S scores of moderately ill or worse decreased to 21% (from 58%) and mean (±SD) YMRS and MADRS improvements were –9.1±12.4 ($P < 0.001$) and –3.6±11.3 ($P < 0.001$), respectively.
Discussion: Preliminary findings from the OL phase of this study suggest that the addition of RLAI to TAU may reduce symptoms for patients with FRBD. Long-term, DB, placebo-controlled data from this ongoing trial will help determine whether BP patients achieving remission with RLAI added to TAU experience a significant delay in the time to relapse compared with those not receiving RLAI.

95. Evaluation of Eszopiclone and Escitalopram Oxalate Co-Therapy in Patients with Generalized Anxiety Disorder and Insomnia

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Background: This study evaluated the efficacy of eszopiclone (ESZ) and concurrent escitalopram oxalate (EO) treatment in patients with insomnia and co-morbid Generalized Anxiety Disorder (GAD).
Methods: Patients meeting DSM-IV-TR criteria for GAD and insomnia received 10 weeks of escitalopram oxalate 10mg and were randomized to nightly eszopiclone 3mg (ESZ+EO; n=294) or placebo (PBO+EO; n=301) for 8 weeks. For the last 2 weeks, ESZ was replaced with single-blind placebo to evaluate discontinuation effects. Additional inclusion criteria were sleep latency ≥ 30 minutes and a total sleep time (TST) ≤ 6.5 hours at least 3 times/week each week over the previous month. Subjective sleep and daytime function were assessed daily. At Weeks 1, 2, 4, 6, 8, and 10, the following clinician assessments were completed: HAM-A, clinical global impression improvement and severity (CGI-I and CGI-S), HAM-D17, insomnia severity index (ISI; Weeks 1,4,8 and 10), and adverse events. The primary endpoint was change from baseline in sleep latency averaged over the double-blind treatment period.
Results: Compared with PBO+EO, ESZ+EO resulted in significantly improved sleep (sleep latency, wake time after sleep onset, TST and number of awakenings) and daytime functioning (daytime alertness, ability to concentrate, and ability to function) at each week and when averaged over the double-blind period ($p<0.05$), with no evidence of tolerance. At Week 8, significantly more ESZ+EO patients had no clinically meaningful insomnia based on the ISI score£7 (47% vs 33%; $p<0.001$). Significant improvements from baseline ($p<0.05$) with ESZ+EO were observed in HAM-A total scores each week, and at Weeks 4-10 when the insomnia item was excluded. CGI-I was significantly improved with ESZ+EO at every timepoint ($p<0.02$), while CGI-S was not different after Week 1. Median time to onset of anxiolytic response was reduced based on HAM-A (29 days for ESZ+EO vs. 32 days for PBO+EO; $p = 0.023$) and CGI-I (18 days for ESZ+EO vs 28 days for PBO+EO; $p=0.052$). HAM-A response (63% vs 49%, respectively; $p<0.05$) and remission (42% vs 36%, respectively; $p=0.09$) at Week 8 were higher in the ESZ+EO group. HAM-D17 scores were also significantly improved from baseline in the ESZ+EO group at all time points ($p<0.004$). At Week 10, after ESZ discontinuation, there was no evidence of rebound insomnia but there were no significant treatment differences for any of the sleep or daytime function measures. In contrast, significant differences between treatments in measures of anxiety and mood were maintained at Week 10. The overall rates of adverse events (AEs) were similar in the two treatment groups (78% for ESZ+EO vs 68% for PBO+EO) with unpleasant taste (24% vs 4%), headache (19% vs 15%), dry mouth (16% vs 9%), somnolence (11% vs 7%) and nausea (10% vs 15%) being the most frequently reported AEs.
Discussion: In this study, eszopiclone/escitalopram oxalate co-administration was well tolerated and associated with significantly improved sleep and daytime function without evidence of tolerance. Significant improvements in anxiety and mood were also observed in the ESZ+EO arm as compared to the PBO+EO arm in patients with GAD and insomnia.

96. Chronic Antidepressants Decrease and Depression Increases the Synaptic Localization of G Protein Gsα in Lipid Rafts: The Tubulin: Gsα Interface as a Putative Target

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Background: Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids and are thought to enhance or inhibit signaling by concentrating or separating signaling molecules. While 5HT2A-Gq signaling is enhanced in lipid rafts, GPCRs coupled to Gs show attenuated signaling in those structures. Gsα migrates from a Triton X-100 (TTX-100) insoluble (raft) membrane domain to a TTX-100 soluble (non-raft) membrane domain in response to chronic treatment with tricyclic or SSRI antidepressants. Gsα, alone among signaling proteins and other membrane-associated proteins, was affected in this manner. These observations have been made in both rats and a clonal (C6 glioma) cell line (Toki et al., 1999 J. Neurochem. 73: 1114-1120; Donati and Rasenick, 2005 Neuropsychopharm. 30: 1238-1245). Chronic antidepressant treatment is known to upregulate cAMP signaling, and recent evidence suggests that Gsα signals more effectively when outside of lipid raft fractions (Allen et al., 2005, Mol. Pharm. 67; 1493-1504).
Methods: Lipid raft Gsα content is determined with sucrose density gradients or with a sequential detergent extraction of a membrane

sample with TTX-100 followed by TTX-114 (a stronger detergent than TTX-100). Using this method, Gsα was shown to migrate out of lipid raft domains and into a more facile association with adenylyl cyclase subsequent to chronic antidepressant treatment. Thus, it was suggested that since antidepressants moved Gsα out of raft domains, a greater portion of Gsα may reside in rafts during depression. To examine this, brain tissue was obtained from the Brain Collection Program of the Maryland Psychiatric Research Center (Baltimore, MD).
Results: Sequential TTX-100 and TTX-114 extractions revealed 2 fold more TTX-100 soluble (non-raft) and 50% less TTX-114 soluble (raft) Gsα in the cerebellum of control (n=14) vs. depressed suicide victims (n=14). Similar results were obtained with cerebral cortex samples. These results suggest that depression is accompanied by movement of Gsα to a membrane domain where it is less likely to couple to adenylyl cyclase and that antidepressants may upregulate Gsα signaling via disruption of the lipid environment in which Gsα is normally ensconced. In an attempt to discern a possible raft-based molecular target for this phenomenon, we focused on tubulin, a known component of rafts in neurons and glia that has also been demonstrated to bind Gsα with a Kd of 115 nM (Wang et.al. J. Biol Chem, 1990, 265:1239 1242). To do this, we explored the effects of antidepressants and microtubule disrupting drugs on the association of tubulin with lipid rafts in C6 rat glioma cells. Fluoxetine treatment significantly reduced the amount of β-tubulin (but not α-tubulin) in lipid rafts. Colchicine treatment indiscriminately and greatly reduced the levels of all isoforms of β-tubulin in lipid rafts and reduced Gsα in lipid rafts by 50% (the same reduction of Gsα is observed with fluoxetine treatment). ILX651 (tasidotin), which, like colchicine, prevents microtubule polymerization, did not remove β-tubulin or Gsα from lipid rafts. No change in the overall content of tubulin was recorded with any of these treatments. Modeling studies suggest that Gsα binds to tubulin in an interface overlapping the GTP binding site. Affinity of tubulin-GTP for Gsα is greater than for tubulin-GDP, and while colchicine activates tubulin GTPase, tasidotin does not.
Discussion: Taken together, these studies suggest that lipid-raft anchoring sites for Gsα are increased in depression and that one effect of antidepressant treatment is to modify those sites, increasing the availability of Gsα to adenylyl cyclase.

97. Effect of Dopamine Transporter (DAT1) VNTR Polymorphism on Eye Tracking Endophenotypes in Schizophrenia

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Sponsor: Robert W. Buchanan

Background: The application of endophenotypes in the genetic dissection of schizophrenia is crucial in reducing clinical and genetic heterogeneity for this syndromal phenotype. Previously we have shown that a dopamine level altering genetic variant in the COMT gene significantly affects predictive pursuit, a specific endophenotype for schizophrenia. In this study, we examined the association between the 40-bp variable number of tandem repeats (VNTR) polymorphism in the dopamine transporter (DAT1) gene and eye tracking endophenotypes in schizophrenia subjects. Two previous studies showed that the homozygous 10-repeat of the VNTR (10/10 genotype) of the DAT1 gene may be functional in changing the availability of dopamine and associated neurocognitive functions in the brain compared to non-10/10 genotypes. We hypothesized that DAT1 VNTR may affect eye tracking performance.
Methods: Maintenance pursuit gain (a global measure of smooth pursuit eye movement) and predictive pursuit gain measures were compared in 89 patients with schizophrenia, 40 unaffected first-degree relatives, and 80 healthy comparison subjects with no family history of schizophrenia, with DAT1 VNTR genotype.
Results: There was a trend difference in maintenance pursuit gain between groups (p=0.07). There was no genotype-by-diagnosis interaction on the maintenance pursuit performance. Predictive pursuit gain was significantly different in patients with schizophrenia compared to their unaffected first-degree relatives and healthy controls (p=0.008). Examination of the effects of being homozygous for the functional 10-repeat allele DAT1 genotype (10/10) vs. non-10-repeat allele genotype showed a significant diagnosis-by-genotype interaction (p=0.016): patients with schizophrenia with the 10/10 genotype fared worse than patients with non-10/10 genotypes in predictive pursuit (p=0.02); while an opposite effect was observed in the healthy control subjects; controls with 10/10 genotype performed better than controls with non-10/10 genotypes (p=0.09, ns). In the relatives the genotype effects were minimal on predictive pursuit performance (p=0.83). Individuals with 10/10 genotype were significantly different in predictive pursuit performance (0.46±0.14, 0.53±0.12, 0.57±0.16) in patients, relatives, and controls, respectively ($F(2, 151)=9.4$, $p<0.001$). The DAT1 gene VNTR genotype alone explained about 3% of the variance in the healthy controls and 6% in schizophrenia patients on their predictive pursuit performance.
Discussion: Our data suggests that this functional DAT1 VNTR polymorphism affects performance on a specific smooth pursuit eye movement measure. Schizophrenia subjects with the DAT1 10/10 genotype performed worse on predictive pursuit gain measures and healthy control subjects with DAT1 10/10 genotype had the best performance. Unaffected first-degree relatives with DAT1 10/10 genotype fared better than schizophrenia subjects, but worse than healthy controls with no family history of schizophrenia. The DAT1 10/10 genotype may increase the availability of dopamine in the brain, which may worsen the performance in schizophrenia patients but enhance the performance in healthy control subjects. This data provides further evidence of a genetic effect on a putative schizophrenia endophenotype.

98. Glucocorticoid Modulation of nAChR α7 Subunit mRNA in Rat Hippocampus

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Sponsor: William Z. Potter

Background: Postmortem binding studies on brain samples from schizophrenic patients (Freedman et al., 1995; Leonard et al., 1998; Martin-Ruiz et al., 2003) and genetic linkage studies (Leonard et al., 2002; Gault et al., 2003) have revealed an involvement of nAChR α7 in schizophrenia. Furthermore, nicotine and subtype selective nicotinic receptor agonists transiently restore cognitive and sensory deficits in schizophrenic patients (Adler et al., 1993; Olincy et al., 1998; Avila et al., 2003; McEvoy et al., 1995; Myers et al., 2004; Sacco et al., 2005; McGehee et al., 1995; George et al., 2001; Smith et al., 2006; Olincy et al., 2006). Elevated circulating glucocorticoid, which can be found in schizophrenic patients, may be associated with cognitive deficits in both patients and animal models and so this study determined if glucocorticoids affect nAChR α7 expression in rat hippocampus.
Methods: Intact female Sprague Dawley rats were treated once daily for four or seven days with dexamethasone (0.01 to 0.1 mg/kg, s.c.) or for four days with corticosterone (1 to 30 mg/kg, s.c.) and dorsal hippocampus removed for analyses. Total RNA was isolated and relative changes in nAChR α7 mRNA determined using real-time RT-PCR.
Results: A maximal 25% reduction in nAChR α7 mRNA was detected following four days of glucocorticoid treatment that increased to 30% with seven days of dosing dexamethasone (0.03 mg/kg ip). In addition, treatment with corticosterone reduced nAChR α7 mRNA levels with the maximal effect of 30% achieved

at 1 mg/kg. Co-administration of a glucocorticoid receptor (GR) antagonist, mifepristone (30 mg/kg s.c.), with 0.1 mg/kg dexamethasone once daily for four days blocked the reduction in nAChR α7 mRNA.
Discussion: These data show that glucocorticoids modulate hippocampal nAChR α7 expression at the mRNA level through a GR specific mechanism and suggest that glucocorticoid modulators may be capable of indirectly affecting nAChR function. GR-specific effects on nAChR expression may have some clinical benefit in disease states associated with cognitive deficit such as schizophrenia.

99. Asenapine: Effect on a Subchronic Phencyclidine-Induced Reversal Learning Deficit in Rats
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Sponsor: Robert H. Roth

Background: Asenapine is a novel psychopharmacologic agent under development for the treatment of schizophrenia and bipolar disorder. Phencyclidine (PCP) can be used to induce cognitive deficits in animal models similar to deficits seen in patients with schizophrenia. We have previously shown that acute administration of PCP in the rat causes cognitive deficits that can be attenuated by pretreatment with asenapine or atypical antipsychotics. In this study, we used subchronic administration of PCP to produce effects that would persist after PCP washout, as marked by a reduction in hippocampal parvalbumin immunoreactivity. Asenapine and atypical antipsychotics were administered on both an acute and a subchronic basis to assess their effects on the PCP-induced reversal learning deficit in the rat.
Methods: Female hooded Lister rats were placed in a Skinner box into which 2 levers could be extended and were trained with rewards to press the active lever, which was identified by a light above the lever. Rats achieving 90% criterion on 3 consecutive days were then trained to reverse this behavior. After training, rats were treated with PCP 2 mg/kg intraperitoneally (ip) twice daily for 7 days, followed by 7 days drug free to allow for washout. For the acute intervention trial, rats were given a single treatment with asenapine (0.025–0.1 mg/kg), risperidone (0.05–0.2 mg/kg), olanzapine (0.5–1.5 mg/kg), or vehicle. For the subchronic intervention trial, rats were given 28 days of treatment with asenapine 0.075 mg/kg subcutaneously twice daily, risperidone 0.2 mg/kg ip once daily, olanzapine 1.5 mg/kg ip once daily, or vehicle. Performance tests were conducted serially during the 28-day protocol, following drug administration on test days. Results are reported as reversal phase scores with PCP plus drug versus PCP plus vehicle.
Results: PCP induced a significant selective reversal learning deficit that was maintained over the 28-day study period. In the acute treatment paradigm, the reversal learning deficit was significantly reduced by single treatments of asenapine 0.05 and 0.075 mg/kg, risperidone 0.2 mg/kg, and olanzapine 1.5 mg/kg ($P<0.05$ to $P<0.001$). With subchronic treatment, the effects of asenapine in attenuating the deficit were statistically significant on test days 3, 7, and 17 ($P<0.01$, $P<0.05$, and $P<0.05$, respectively); for test days 21 and 28, $P=0.07$ and $P=0.06$. Risperidone effects were significant on test days 3, 7, and 28 ($P<0.01$, $P<0.05$, and $P<0.05$); for test days 17 and 21, $P=0.06$ and $P>0.1$. Olanzapine effects were significant on test days 3 and 17 (both $P<0.05$); for test days 7, 21, and 28, $P=0.055$, $P=0.08$, and $P>0.1$.
Discussion: Subchronic administration of PCP induced an enduring deficit in reversal learning in the rat. In the acute study, the effects of asenapine in attenuating this cognitive deficit were dose-related. In the subchronic study, the effects of asenapine showed no substantial decline over time. These results with asenapine were similar to those produced with risperidone and olanzapine. This improvement in cognitive function seen in an animal model suggests potential clinical relevance in the management of schizophrenia.

100. Assessment of Akathisia in Acute Schizophrenia and Schizoaffective Disorder Patients: Results from a Pooled Analysis of 5 Short-Term, Placebo-Controlled, Double-Blind Studies with Aripiprazole
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Background: Although akathisia is usually associated with first generation antipsychotics (FGAs), it remains a challenge in routine psychiatric practice, despite the widespread use of second generation antipsychotics (SGAs). Recently reported prevalence rates cites 11.5% of akathisia in a sample of psychiatric inpatients and 15% in outpatient schizophrenia patients. This analysis was performed to quantify and qualify clinical characteristics of akathisia in schizophrenia or schizoaffective disorder patients experiencing an acute relapse who were randomized to receive aripiprazole (Ari), or placebo (Pbo) in 5 pooled short term trials.
Methods: A post hoc analysis of the safety dataset was conducted to assess clinical aspects of akathisia in five 4 or 6 weeks, double-blind, randomized trials comparing aripiprazole (2, 5, 10, 15, 20, 30mg/day) to placebo. The following akathisia parameters were assessed: incidence, time to onset, duration and severity of symptoms, concomitant use of benzodiazepine (BZD), and scores on the Barnes Akathisia Rating Scale (BARS).
Results: A total of 1,635 patients was included in this analysis (Ari: n=1170; Pbo: n=465). Akathisia was reported by 9% of the Ari-treated patients and 6% of those receiving Pbo. Among those reporting akathisia, more patients receiving Ari (83%, n=86) reported this AEs within the first 2 weeks of the trials when compared to Pbo (69%, n=20). The mean and median duration of akathisia was generally low in both groups (Mean: Ari=12.5 days and Pbo=4.2 days; Median, Ari=5.0 days and Pbo=1.5 days). Interestingly, the severity of akathisia was similar among patients receiving Ari or Pbo, with the majority of patients reporting this EAs as mild or moderate (Ari: 91% ; Pbo: 93%). Furthermore, under double-blind conditions, relationship to study drug as assessed by the investigators was described as probable or certain for 19% for the Ari group and 14% for the Pbo group. Only 4 patients discontinued the study due to akathisia in the Ari group (0.3%) while none did so in the Pbo group. The use of BZD among patients reporting akathisia was high in both treatment arms and somewhat higher in the Ari group (95%) than in the Pbo one (86%). Mean changes from baseline to endpoint in the BARS scores were not statistically different between Ari versus Pbo groups (p=0.2). Finally, the percentage of patients reporting akathisia at endpoint (BARS Item 4 ≥ 2) was similar between Ari- (16%) and Pbo-treated patients (14%). When the same analyses were performed excluding the two lower doses of aripiprazole (2 and 5 mg), no differences were found in the variables analyzed.
Discussion: Extrapyramidal symptoms, including akathisia, are more commonly associated with the use of FGAs; although in lower rates, akathisia is also reported by patients using SGAs. In this analysis, in the aripiprazole and placebo groups, akathisia appeared to occur early in treatment, be time-limited, and associated with high rates of concomitant benzodiazepine usage. Additionally, most cases of akathisia were reported as mild to moderate and rarely associated with treatment discontinuation.

101. The Metabolic Profile of Bifeprunox in the Treatment of Patients with Schizophrenia
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Background: Objectives: Antipsychotics have been associated with metabolic adverse events, including hyperglycemia, dyslipidemia, and

weight gain. The purpose of this analysis was to examine the metabolic effects of bifeprunox, compared to placebo and active control medications, in patients with acute and stable schizophrenia.
Methods: Metabolic data was compiled from four 6-week randomized, double-blind, placebo-controlled, active-referenced studies of bifeprunox treatment for acute exacerbation of schizophrenia. Metabolic data was also examined from a 6-month randomized, double-blind, placebo-controlled, study of bifeprunox for chronic stable schizophrenia. From the 6-week studies, the pooled treatment groups were bifeprunox (n=1050), placebo (n=469), haloperidol (n=52), risperidone (n=274) or olanzapine (n=150). In the 26-week study, patients received either bifeprunox (n=331) or placebo (n=166). Metabolic evaluations included body weight, body mass index (BMI), plasma glucose and lipid profiles. Fasting glucose and lipid values were assessed in one 6-week study and in the 6-month study, with triglyceride:HDL cholesterol ratio (TG:HDL) calculated as a marker of insulin resistance. Changes from baseline to last assessment were analyzed and compared between treatments.
Results: In the 6-week studies, mean weight decreases from baseline occurred in groups treated with bifeprunox (-0.8 kg) and placebo (-0.1 kg), whereas groups treated with olanzapine (+2.4 kg) and risperidone (+1.7 kg) experienced mean weight increases. Mean body weight decreases in the 26-week study were -1.1 kg in the bifeprunox group compared to -0.5 kg with placebo. Bifeprunox-related weight decreases exceeded placebo-related reductions, independent of the occurrence of nausea and vomiting. In the 6-week studies, mean weight decrease occurred on bifeprunox in all but the lowest BMI group (<18.5 kg/m2), with the largest mean weight loss in the highest BMI group (>30 kg/m2). Mean fasting glucose values in patients receiving bifeprunox decreased by 2% (-0.1 mmol/L) from baseline in the 6-week study, compared to a 6% increase (+0.3 mmol/L) for placebo. In the 26-week study, there was an increase in mean fasting glucose in both bifeprunox-treated (5%; +0.2 mmol/L) and placebo-treated groups (1%; +0.1 mmol/L) compared to baseline. Mean fasting total cholesterol (TC) decreased from baseline in the 6-week study (8%; -0.40 mmol/L for bifeprunox versus 6%; -0.32 mmol/L for placebo), and the 26-week study (6%; -0.31 mmol/L for bifeprunox versus 2%; -0.08 mmol/L for placebo). In the 6-week study, mean fasting TG values decreased in the bifeprunox-treated group by 24% (-0.44 mmol/L), with similar decreases in TG:HDL; the placebo-treated group had similar decreases in TG (20%; -0.38 mmol/L) and TG:HDL (17%). In the 6-month study, fasting TG values decreased in the bifeprunox group by 19% (-0.32 mmol/L) versus 3% in the placebo group (-0.05 mmol/L), with a 23% decrease in TG:HDL in the bifeprunox group in the 6-month study, compared to a 4% decrease in patients receiving placebo.
Discussion: In this analysis of placebo and active-controlled studies, decreases in body weight and improvement in lipid profiles were observed in patients treated with bifeprunox, with minimal changes in plasma glucose. Reductions in weight, TG, TC and TG:HDL were sustained in patients receiving 6-months of treatment. The metabolic profile of bifeprunox suggests that it may have safety advantages compared to other antipsychotics in the treatment of schizophrenia.

102. Relationship of Plasma Cortisol to Adiposity and Metabolic Indices in Schizophrenia Patients

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Background: Recent reports have postulated that stress-related increases in circulating cortisol levels could account for increases in adiposity as well as insulin resistance and related dyslipidemia observed in patients with schizophrenia. This hypothesis has been suggested for unmedicated, drug-naïve patients and extended to treated patients.
Methods: To test this hypothesis in a group of treated schizophrenia patients, we analyzed the association between fasting early morning plasma cortisol levels and direct measures of adiposity, gold-standard measures of insulin sensitivity, and fasting lipid levels, using a sample size (n=77) with good power to detect moderate or larger effects. Magnetic Resonance Imaging (MRI) was used to quantify adipose tissue, and the hyperinsulinemic, euglycemic clamp technique with stable isotopomer tracers was used to determine whole body and tissue-specific insulin sensitivity.
Results: No significant association was detected between plasma cortisol and MRI-measured visceral adipose tissue area ($F[1,46]=0.37$, $p=0.55$), subcutaneous adipose tissue area ($F[1,46]=0.36$, $p=0.55$), or combined visceral and subcutaneous adipose tissue ($F[1,46]=0.003$, $p=0.95$). Similarly, there was no significant association between plasma cortisol and clamp-derived measures of whole body insulin sensitivity (glucose disposal rate, $F[1,42]=0.63$, $p=0.43$), or tissue-related insulin sensitivity (hepatic: glucose rate of appearance, $F[1,66]=0.033$, $p=0.86$; skeletal muscle: glucose rate of disappearance, $F[1,66]=0.33$,$p=0.57$; adipose tissue: glycerol rate of appearance, $F[1,67]=1.71$, $p=0.20$). Finally, no significant relationship was detected between plasma cortisol and other metabolic indices, including fasting glucose level ($F[1,75]=1.41$, $p=0.24$), fasting insulin level ($F[1,75]=0.75$, $p=0.39$), fasting triglyceride level ($F[1,73]=2.11$, $p=0.15$), fasting total cholesterol level ($F[1,73]=0.025$, $p=0.87$) fasting LDL level ($F[1,72]=0.09$, $p=0.76$), or fasting HDL level ($F[1,73]=2.98$, $p=0.09$).
Discussion: These results do not support the hypothesis that increased adiposity or impairments in glucose and lipid metabolism are related to plasma cortisol levels in chronically treated schizophrenia patients.

103. Long-Term Aripiprazole Treatment Alters Smoking Behavior in Chronic Schizophrenia Patients: A Pilot Study

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Sponsor: Daniel E. Casey

Background: As a group, patients with schizophrenia have three times the smoking prevalence compared to the general population, and twofold greater cardiovascular mortality. Baseline data from the CATIE Schizophrenia Trial indicate that the current prevalence of smoking among US schizophrenia patients is 65%. Multiple neurobiological hypotheses have been generated to explain this high smoking prevalence, with a primary focus being the effects of nicotine on cholinergic and dopaminergic pathways. Dopaminergic deficits in reward pathways, in particular, have been advanced as a common mechanism to explain the propensity for abuse of various substances among patients with schizophrenia. The development of antipsychotics with intrinsic dopaminergic partial agonism offers an opportunity to examine this hypothesis by studying the effects of these agents on addiction in schizophrenia patients. Smoking is chosen as a model for study due to its public health importance, the fact that it is a daily habit, and the availability of means to biologically quantify changes in smoking behavior.
Methods: 15 stable outpatient subjects with schizophrenia (12M/3F, mean age 47.4 years, mean duration of illness 23.1 years, mean PANSS total score 62.7), underwent an 8-week open-label switch to the dopamine partial agonist aripiprazole, with 8 subjects followed in a 20-week extension. Subjects were paid a nominal amount for each visit ($20). There was no inducement to change one's smoking behavior, and no formal program to support smoking reduction. Primary outcome measures included the Fagerstrom Test for Nicotine Dependence, and measurement of the nicotine metabolite cotinine ($t^1/_3$ 16 hours) in saliva at weeks 0, 4, 8 and 28.
Results: 15 subjects completed the initial 8-week study, and 8 completed the 20-week extension. There were no significant between-group baseline demographic differences between subjects who completed the 8-week study, and those who completed 28 weeks. Mean endpoint aripiprazole dose was 15.71 mg for those who completed 8

weeks, and 18.75 mg for those who continued through week 28. Salivary cotinine changed nonsignificantly from baseline (388.6 ± 363.9 ng/ml) to the week 8 endpoint (379.2 ± 347.9 ng/ml) in the initial sample of 15 subjects. Among the cohort of 8 subjects who continued in the 20-week extension, there was a significant decrease in salivary cotinine from baseline (471.9 ± 447.1 ng/ml) to the week 28 endpoint (255.2 ± 105.9 ng/ml) (p=.036), with 3 of 8 subjects experiencing greater than 50% reductions in cotinine levels. While the switch to aripiprazole decreased smoking behavior by week 28, as measured by salivary cotinine, there was no change in subjective drive, as measured by the Fagerstrom score. Decreases in salivary cotinine did not correlate with changes in PANSS scores, or ratings of akathisia or other measures of extrapyramidal side effects.
Discussion: Dopaminergic mechanisms may be important mediators of the smoking drive in patients with schizophrenia. This pilot study indicates that switching schizophrenia patients to a dopamine partial agonist, aripiprazole, was associated with decreased smoking behavior, and thus may prove useful in a harm reduction model aimed at minimizing smoking-related health risks.

104. Deficit in Sustained Activity in the DLPFC During Working Memory Maintenance in Schizophrenia

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Background: Although the vast majority of functional magnetic resonance imaging (fMRI) studies in schizophrenia (SZ) research have demonstrated abnormalities in the dorsolateral prefrontal cortex (DLPFC) during working memory (WM), there has been a paucity of event-related studies examining the temporal dynamics of DLPFC activity associated with WM performance in SZ. The identification of deficits in specific component processes is a crucial step in understanding the physiologic mechanism of dysfunction in SZ. Doing so would implicate a distinct set of neural regions and processes, as well as cellular and molecular targets, for the development of animal models of cognitive dysfunction and novel treatment strategies. The focus of this study is active maintenance—the sustained representation of information required to efficiently guide decisions. The classic electrophysiological studies from Fuster and Goldman-Rakic have demonstrated that the neural basis of active maintenance is sustained neural activity across the delay period during WM in the DLPFC. The determination of whether the DLPFC in SZ retains this capacity for sustained activity would have significant impact on our understanding of the neural basis of this condition. Here we present preliminary results of an event related fMRI study utilizing BOLD time series analysis of DLPFC activity of patients with SZ and healthy control subjects while they engaged in a WM task.
Methods: Individuals with SZ and healthy controls (C) underwent fMRI in a 3T scanner while performing a delayed response task in which the subject processes cue and probe faces. During cue, the subject is shown a face for 1 sec. A cross hair then appears for 15 seconds in the delay period. At the end of the delay, a probe face appears prompting a response from the subject. There are two conditions. The appearance of a white cross hair at the beginning of the delay period requires the subject to maintain the image of the cue face across the delay in order to make a face identity match response with the probe face—the visual WM condition. The appearance of a red cross requires the subject to make a determination of the gender of the probe face when it appears—the probe gender identification (PGID) condition. Note that in the latter condition, there is no need to maintain any cue related information because the response is solely dependent on the probe visual properties. FMRI analysis is conducted in the subject's untransformed native space and functional ROIs are selected based on significant cue related activation in the DLPFC. The main dependent measure is the degree of between condition enhancement of the BOLD time series in the DLPFC across the delay period.
Results: Behavioral performance was good for both groups and did not differ between groups. Despite equivalent performance, there is a notable difference across groups in the pattern of DLPFC activity during the delay period. Control subjects showed enhancement in the delay period signal in the WM condition compared to the PGID condition. In contrast, SZ subjects did not show this enhancement of DLPFC activity.
Discussion: This result demonstrates a DLPFC deficit in supporting sustained neural activity that may in and of itself serve as a target for treatment development aimed at remediating cognitive deficits in schizophrenia. It also delineates a set of candidate physiological mechanisms underlying this aspect of impaired cognition in schizophrenia, including synchronous oscillations in thalamocortical neuronal assemblies and neurotransmitter systems that modulate this activity.

105. Sertindole Reverses Cognitive Impairment Induced by PCP in Two Rat Models and Enhances Frontal Cortical NMDA Function in vitro

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Background: Sertindole (SER) is a novel antipsychotic drug (APD) with potent effects on D2, 5-HT2A,2C,6 and α1-adrenergic receptors but no effects on histaminergic and muscarinic receptors. SER does not cause extrapyramidal symptoms in patients in the recommended dose range and does not impair cognitive function in animals at clinically relevant plasma concentrations. Here we examined whether SER and risperidone (RISP) may improve cognitive functioning in rats impaired by acute or subchronic administration of the NMDA receptor antagonist phencyclidine (PCP). Their effects in vitro on NMDA-induced responses in pyramidal cells of the rat medial prefrontal cortex (mPFC) were also examined using electrophysiological methods.
Methods: Reversal of PCP-impaired water maze performance in male Wistar rats: PCP was injected daily at a dose of 1.3 mg/kg (SC), i.e. during 3 days before the first acquisition trial and during 3 days of acquisition, 30 min before the first trial. Both SER and RISP were injected SC 1 h before the first trial. Methods are further described by Didriksen et al, Eur J Pharmacol, 542, 108, 2006. Reversal of PCP-impaired attentional set shifting in male Long-Evans rats (Rodefer et al, Eur J Neurosci, 21, 1070, 2005): PCP 5 mg/kg was injected IP BID for 1 week, followed by 10 days withdrawal. Attentional set shifting was studied after acute treatment with SER (PO, 2 hrs before the test) or RISP (IP, 30 min before). PCP specifically impairs the acquisition of extradimensional shifting, leaving other tasks unaffected. Effect of SER on NMDA-induced currents in pyramidal cells of the mPFC: Rat mPFC brain slices were used for electrophysiological intracellular recording (Jardemark et al, Int J Neuropsychopharmacol 8, 157, 2005). SER was administered via bath perfusion and NMDA (5-20 μM) was similarly applied to induce inward currents. The effect of SER on the NMDA-induced current was calculated by dividing the NMDA-induced current after bath applications of the drug by the control NMDA-induced current.
Results: SER (0.63-2.5 mg/kg) reversed the PCP-induced impairment of spatial learning in the water maze on test day 2 and 3, as measured by decreased swimming distance and number of non-finder trials (i.e. the fraction of trials in which the rat failed to find the submerged platform within the total trial period of 1 minute). RISP (0.04-0.16 mg/kg) had variable PCP-reversing effects. In the attentional set shifting model SER (0.63-2.5 mg/kg) dose-dependently reversed the impairment of extradimensional shifting induced by previous PCP treatment, while RISP (0.03-0.3 mg/kg) induced a modest but not significant reversal. Electrophysiologically, SER in similarity with all

2nd, but not 1st generation APDs was found to potentiate NMDA-induced responses in the pyramidal cells of the mPFC.
Discussion: The results show that SER exerts a robust cognitive-improving effect in two rat models of PCP-induced impairment of relevance for the cognitive deficits in schizophrenia. RISP showed some effect in the water maze model (depending on hippocampus), but failed to show significant effect in the set shifting model (depending on frontal cortex). The mechanisms behind the different profiles of SER and RISP remain to be clarified, but potentially the affinities for 5-HT6 receptors (high for SER, low for RISP) may be involved, as this receptor has been associated with cognitive performance in the literature. Both SER and RISP are potent 5-HT2A receptor antagonists and share enhancement of NMDA receptor-mediated transmission in the mPFC with 5-HT2A antagonists. Thus NMDA enhancement may be part of the mechanism involved in reversal of cognitive deficits, but cannot fully explain the differential profile of SER and RISP.

106. Smoking, Symptom Severity, Cognition and Auditory Gating
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Background: P50 auditory gating deficit is one of the best established biological traits associated with schizophrenia. Tobacco smoking improves P50 sensory gating, albeit temporarily., and up to 80% of patients with schizophrenia smoke an average of 30 cigarettes per day and extract 50% more nicotine than other smokers. Increased activation of $\alpha 7$ nicotinic acetylcholine receptors appears to account for this effect. Although nicotine improves attention, its effects on working and visuospatial memory are debatable. This study explored the effects of smoking and illness severity on P50 Cz and superior temporal gyrus (STG) M50 gating and neurocognition in chronic schizophrenia. This study is part of a larger NIH R01 data set that examines the cortical basis of schizophrenia gating deficit.
Methods: Sixty-nine patients and 58 controls participated in the study (among them 52 were smokers and 75 were non-smokers). All subjects were administered tests assessing IQ, attention and working memory. Symptom severity in the schizophrenia group was measured with the PANSS. All patients were clinically stable on a single antipsychotic medication for 3 months. Smokers were asked to refrain from smoking for at least one hour before examination. Simultaneous EEG and MEG data were collected using a whole-head Neuromag 122-channel biomagnetometer while patients were administered the standard paired-click paradigm. P50 Cz and left and right M50 STG gating ratio scores were computed (50 ms response to second click divided by 50 ms response to first click). Schizophrenia patients were divided into a low and high symptom severity groups (LS and HS respectively), based on a mean PANSS score of 60. This resulted in a LS group of 35 subjects and a HS group of 34 subjects.
Results: ANOVA on P50 gating ratios found a main effect of diagnosis, $F(1, 123) = 5.369$, $p < 0.05$. Controls (0.459) had better P50 gating than patients (0.589). There were no significant findings for an effect of smoking status or neurocognition on P50. Controls (0.513) showed better M50 STG gating than patients with schizophrenia (0.593), $F(1, 112) = 5.192$, $p < 0.05$. In addition, smokers (0.517) had better sensory gating than non-smokers (0.588), $F(1, 112) = 4.041$, $p < 0.05$. A significant interaction among hemisphere, symptom severity, and smoking status was found, $F(1, 59) = 0.027$, $p < 0.05$. Among the HS group, smokers ($N = 20$, 0.52 ± 0.20) had better right M50 STG gating than non-smokers ($N = 11$, 0.70 ± 0.18), $t(29) = -2.49$, $p < 0.05$, and smokers had a marginally better right hemisphere (0.52 ± 0.20) than left hemisphere (0.62 ± 0.24) M50 gating, $t(19) = 2.09$, $p = 0.051$. However, non-smokers had a better left (0.55 ± 0.15) than right (0.70 ± 0.18) M50 sensory gating, $t(10) = -3.62$, $p < 0.01$. Finally, smokers had lower Shipley IQ scores, $F(1,34) = 6.02$, $p < 0.05$, but there was no significant relation between smoking status or neurocognition and M50.
Discussion: An effect of smoking on P50 gating was not observed. The effect of smoking on P50 has been reported to be short (less than 20 minutes) and likely explains current findings. Smoking status was related to M50 STG gating, suggesting a chronic rather than acute effect. Interestingly, smoking improves right more than left M50 STG gating implying hemispheric differences the response to nicotine. The lack of relationship between smoking and cognition was unexpected. Analyses examining the relationship of serum levels of nicotine and cotinine to P50 and M50 gating and cognition will expand current findings.

107. Effects of Risperidone Microspheres on Polypharmacy and Cognitive Function
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Sponsor: Richard S.E. Keefe

Background: When a patient with a psychotic disorder has an incomplete therapeutic response to an antipsychotic, the treating clinician may add a second antipsychotic or adjunctive medication (mood stabilizer, antidepressant, etc.) in an effort to better control the psychopathology. However, incomplete therapeutic response has been shown to be associated with poor medication adherence, a problem that is not corrected by additional medications. We examined whether assured antipsychotic treatment through risperidone microspheres allowed for a discontinuation of other antipsychotics and adjunctive medications. We also examined whether continued treatment was associated with improvement in cognitive function.
Methods: We switched patients with a psychotic disorder and incomplete adherence and/or polypharmacy to treatment with Risperdal Consta. Over a one year follow-up, we attempted to reduce polypharmacy by reducing the number of antipsychotics, adjunctive medications or both, and assessed clinical course (Brief Psychiatric Rating Scale) and change in cognitive function (Brief Assessment of Cognition in Schizophrenia).
Results: Of 54 subjects, 30 completed one year, 9 completed 6 months, 9 completed 4 months, and 6 completed 2 months. A total of 27 (50%) had a reduction in the number of antipsychotics and/or adjunctive medications. Analysis of time to dropout by whether the subject had a reduction in number of medications indicated no differential dropout rate. Improvement in psychopathology (BPRS ratings) reached maximum at 6 months, with no significant change thereafter. Components of cognitive function (BACS) showed gradual improvement over the 12 months, reaching significance for verbal memory and symbol coding.
Discussion: These results support the hypothesis that assured treatment with risperidone microspheres may permit a reduction in polypharmacy. Subjects continuing on risperidone microspheres also showed improvements in cognitive function.

108. 4Tesla Proton-Magnetic Resonance Spectroscopy (1H-MRS) Longitudinal Study of Early Schizophrenia: Effects on N-Acetyl Aspartate and Glutamate
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Sponsor: Jan Fawcett

Background: Glutamate-mediated excitotoxicity, secondary to NMDA-hypofunction has been postulated to account for the symptoms, cognitive defects, and poor outcome of schizophrenia. Many 1H-MRS cross-sectional studies using mostly clinical scanners (e.g. 1.5T), have found lower N-acetylaspartate (NAA, a marker of neu-

ronal viability), in chronically-treated schizophrenia patients (Steen, Neuropsychoph, 2005, for meta-analysis). The few studies early in the illness (Theberge, AJP, 2002; Tibbo, AJP, 2004; Tebartz van Elst, Biol Psych, 2005), at higher field strength and shorter time-to-echo (TE), found normal frontal NAA and elevated glutamate (Glu), glutamine (Gln) or both (Glx). We used single voxel 1H-MRS at 4T with short TE, to study NAA, Glu and Gln in young schizophrenia patients before and after antipsychotic treatment and healthy controls.
Methods: Fifteen early schizophrenia patients with a history of minimal medication exposure (<3 wks lifetime), were scanned before antipsychotic medications were started, and then following treatment at 1, 6 and 12 months. Treatment with antipsychotic drugs was standardized according to an explicit algorithm. The regions of interest were voxels of mostly homogeneous gray matter in the anterior cingulate (8 cc), left medial thalamus (1.5 cc) and left prefrontal white matter (1.5cc). We used spectroscopic acquisition and analysis methods developed at the University of Western Ontario (Bartha, MRM, 2000). Briefly, spectra were acquired by using STEAM (TR=2000 msec, TE=20 msec, TM=30 msec, dwell time=500 sec, 256 water-suppressed and 16 water-unsuppressed averages) with a 4-T scanner (Varian, Palo Alto, Calif.). Water suppression was achieved by using three chemical-shift-selective pulses. Spectra were analyzed by using curve-fitting software and normalized to water-yielding quantification of NAA, choline (Cho), creatine (Cre), glutamate (Glu) and glutamine (Gln) (Bartha, MRM, 2000). Metabolite values were partial volume corrected for proportion of CSF in the voxel. A group of 10 healthy volunteers was studied once with 1H-MRS.
Results: Only NAA and Glu were reduced in anterior cingulate of schizophrenia subjects at baseline (t=2.19, p=0.04 and t=2.38, p=0.03, respectively). None of the other metabolites differed between the groups in the other regions.Follow-up scans in the schizophrenia group failed to detect changes over time with treatment in any of the metabolites studied.
Discussion: These findings suggest that anterior cingulate NAA reductions in schizophrenia are not a result of chronicity or antipsychotic medication exposure; furthermore, because we used a very short TE (20 ms), this result is relatively insensitive to possible changes in T2. Finally, concurrent reductions in anterior cingulate Glu are consistent with NMDA-mediated excitotoxicity.

109. Synch Before You Speak: Auditory Hallucinations in Schizophrenia

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Background: Synchronization of neural activity preceding self-generated actions may reflect the operation of forward model, which acts to dampen sensations resulting from those actions. If true, pre-action synchrony should be related to subsequent sensory suppression. Deficits in this mechanism may be characteristic of schizophrenia and related to positive symptoms, such as auditory hallucinations. If true, schizophrenia patients should have reduced neural synchrony preceding movements, especially patients with severe hallucinations.
Methods: In 24 patients with schizophrenia or schizo-affective disorder and 25 healthy controls, we related pre-speech neural synchrony to subsequent auditory cortical responsiveness to the spoken sound, compared pre-speech synchrony in schizophrenia patients and healthy controls, and related pre-speech synchrony to auditory hallucination severity in patients. To assess neural synchrony, phase coherence of single-trial electroencephalography (EEG) preceding talking was calculated, at a single site, across repeated trials. To assess auditory cortical suppression, the N1 event-related brain potential (ERP) to speech sound onset during Talking and Listening were compared.
Results: In healthy controls, pre-speech neural synchrony was related to subsequent suppression of responsiveness to the spoken sound as reflected in reduction of N1 during Talking relative to Listening. There was greater pre-speech synchrony in controls than in patients, especially those with severe auditory hallucinations.
Discussion: These data suggest EEG synchrony preceding speech reflects the action of a forward model system, which dampens auditory responsiveness to self-generated speech and is deficient in patients who hallucinate.

110. Altered Homer Protein Expression in the Limbic System Following Prenatal Stress: Implications for the Etiology of Schizophrenia

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Background: The etiology and neurobiological underpinnings of schizophrenia are largely unknown but it is generally believed to result from a combination of genetic and environmental factors. Further, several lines of evidence implicate alterations in glutamatergic functioning in schizophrenia. A number of animal models have been developed to elucidate factors contributing to the expression of schizophrenic symptoms. Prenatal stress is a widely employed model of schizophrenia that implicates alternations in neurodevelopmental trajectories in the etiology of schizophrenia. Conversely, the Homer1 (a scaffolding protein involved in integration of postsynaptic glutamate receptor signals) null mouse has recently been posited as a potential model of psychoses implicating genetic contributions to vulnerability to schizophrenia. These models exhibit a remarkable number of behavioral and neurochemical commonalities, including elevated locomotor responsiveness to stimulant drugs, elevated anxiety/stress responses, sensory-motor deficits (e.g. abnormal pre-pulse inhibition), learning deficits, and elevated glutamatergic responses to stimulant drugs. Accordingly, we asked are there abnormalities in Homer1 proteins in the limbic system of prenatally-stressed rats?
Methods: Pregnant Sprague-Dawley dams were either left undisturbed or subjected to a 30-min restraint stress 3 times per day for the last 7 days of gestation. Offspring and dams were left undisturbed until weaning (age 21-22 d), at which point offspring were sacrificed by decapitation and tissue samples were collected from several limbic structures. Immunoblotting for total protein content was conducted using antibodies against Homer 1b/c, Homer 1a, and Homer 2a/b and standard immunoblotting procedures.
Results: Relative to control rats, rats subjected to prenatal stress exhibited elevated levels of Homer1a in the prefrontal cortex and elevated levels of Homer2 in the hippocampus.
Discussion: As predicted, prenatally-stressed rats had alterations in the expression of specific Homer protein isoforms in limbic brain regions. However, the relationship between Homer proteins and phenotypic indices of psychoses appears to be complex given that we observed elevations in Homer proteins despite the similar phenotypes observed in prenatally-stressed and Homer1 null rodents. Nevertheless, the present data are consistent with earlier preclinical data implicating Homer1 isoforms in anxiety and "psychotic-like" behaviors, as well as clinical data indicating a single nucleotide polymorphism in Homer1 in patients with schizophrenia. As the current analyses were performed in early adolescence, it is suggested that abnormalities in Homer protein expression may serve as a predisposing factor to the expression of schizophrenic symptoms which typically manifest in late adolescence to early adulthood. This work was supported by a Santa Barbara Cottage Hospital Research Award to TEK and NARSAD Young Investigator Awards to TEK and KKS.

111. Genetic Variation Influences Cognitive Decline in Schizophrenia

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Approximately 50% of patients with schizophrenia (SZ) are characterized as "deteriorating", with an IQ decline of > 10

points from premorbid IQ, while another 50% of patients do not demonstrate significant intellectual decline (Reichenberg et al. 2005; Weickert et al. 2000). It is likely that genetic influences play a role in determining the severity of the decline, yet to date there have been no studies to identify specific genes that may differentiate these heterogeneous cognitive profiles. Recently, a growing body of evidence suggests that the gene coding for dysbindin-1 (DTNBP1) might influence intellectual decline in SZ, including recent work by our group that found that DTNBP1 exerted a generalized effect on cognition (Burdick et al. 2006). Thus, we assessed the relationship between DTNBP1 and intellectual decline in SZ.
Methods: We assessed cognitive decline in 183 Caucasian SZ patients using a proxy measure of premorbid IQ with which current general cognitive ability (g) was compared. The composite for g was calculated as the first unrotated factor of a principal components analysis that included the following tests: WAIS-R-Digit Span; Continuous Performance Test-Identical Pairs; California Verbal Learning Test; Fluency; and Trails A&B. We then tested for a relationship between intellectual decline and the 6-locus DTNBP1 risk haplotype (consisting of SNPs: rs909706, rs1018381, rs2619522, rs760761, and rs1011313) which was identified in previous work by our group (Funke et al. 2004).
Results: We found that patients who carry the CTCTAC risk haplotype (n=35) demonstrated a significantly greater decline in IQ (residual mean change=13.5+13.6) as compared with non-carriers (n=148) (residual mean change=8.7+12.4) (p=0.05; Eta2=0.22). In the group of deteriorating patients, the risk haplotype had a frequency of 24%, as compared with a frequency of only 15% in the non-deteriorating group.
Discussion: These data suggest that DTNBP1 influences the severity of intellectual decline in schizophrenia and may represent one underlying cause for heterogeneity in cognitive course. The mechanism underlying this effect of DTNBP1 genotype on cognition is unknown, although its broad distribution in brain, along with reported reductions of DTNBP1 expression in regions critical to cognitive function (Weickert et al.2004), suggests that intellectual decline may be related to decreased DTNBP1 expression. This is supported by preliminary data from a knockdown model demonstrating that reduced DTNBP1 expression results in dysfunction within the glutamatergic system (Numakawa et al. 2004), a system believed to be related to cognitive function and disrupted in schizophrenia.

112. *RGS4* Polymorphisms and Cognitive Function in Multiplex Schizophrenia Families

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Sponsor: Past Travel Awardee, PMRTP, 2004

Background: The Gene encoding the Regulator of G-protein Signaling, subtype 4 (RGS4) has been implicated in the etiology of schizophrenia. RGS4 polymorphisms were associated with volumetric alterations of the dorsolateral prefrontal cortex (DLPFC) in first episode schizophrenia patients. DLPFC has been proposed as a key region in the regulation of cognitive functions, such as working memory and attention that are implicated in the etiology of schizophrenia. Our group further observed that many of these cognitive functions were heritable and distinguished patients from their relatives and healthy controls. In this study, we examined whether these cognitive functions were associated with RGS4 polymorphisms in multiplex multigenerational (MM) Caucasian families with schizophrenia.
Methods: We have analyzed the data from 37 MM families recruited under the Neurobehavioral Family Study of Schizophrenia. The web-based Computerized Neurocognitive Battery (CNB) was administered to all study participants. The CNB evaluates speed (response time) and accuracy of performance on abstraction and mental flexibility, attention, verbal, spatial and face memory, and spatial ability. Based on intensive sequencing and linkage disequilibrium (LD) analysis, we selected 9 'tag' SNPs that represent common polymorphisms at RGS4, namely rs10917670 (SNP1), rs951436 (SNP4), rs951439 (SNP7), ss35522247, ss35522248, rs6427711, rs2661319 (SNP18), rs10799897 and rs10759. These SNPs were genotyped among 471 members of our MM schizophrenia families using SNaPSHOT assay. Measured genotype analyses accounting for family relationships were performed in SOLAR. rs951436 and rs10759 were not included in the analyses because the genotype for these markers and CNB data was not available on all subjects.
Results: In relation to CNB scores, we found that SNPs rs10917670 (SNP1) and rs951439 (SNP7) were significantly associated with speed (p=0.0003) and accuracy (p=0.03) on face memory and speed on verbal memory (p=0.02) tasks. Association of facial memory speed survived a highly conservative Bonferroni correction, the significance of association for response time on facial memory persists (p=0.039) (19 traits with 7 markers, total tests 133). There were suggestive associations with accuracy of verbal memory (p=0.09) and semantic memory (p=0.18). In addition, there were significant associations of abstraction/mental flexibility (p=0.02) and verbal memory accuracy scores (p=0.03) for SNP 9, and spatial memory accuracy (p=0.02) for SNP 18 (rs2661319).
Discussion: Our observations suggest that RGS4 polymorphisms are associated with variations in cognitive functions, especially memory, which are frequently reported in schizophrenia patients and their relatives. These cognitive functions are regulated by a distributed neural network where DLPFC plays a critical role.

113. Dysbindin-1 is a Synaptic and Microtubular Protein that Binds Brain Snapin

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Sponsor: Steven E. Arnold

Background: Genetic variation in the gene encoding dysbindin-1 has frequently been associated with schizophrenia. Recent studies indicate that the protein also plays a general role in cognition, perhaps by affecting synaptic glutamate release. How dysbindin-1 might affect such release is unknown without discovery of the protein's neuronal binding partners and its subcellular locus of action.
Methods: Tissue fractionation and immunoprecipitation, Western blotting, and immunohistochemistry were used to examine the regional and subcellular localization and expression of dysbindin-1, snapin, NMDAR1ζ, actin, PSD-95, Rab3, and synaptophysin in mouse and postmortem human brain tissue. Immunoelectron microscopy was used to examine the ultrastructural localization of dysbindin-1 in mouse and macaque hippocampus.
Results: We demonstrate here that snapin is a binding partner of dysbindin-1 in vitro and in the brain. Tissue fractionation of whole mouse brains and human hippocampal formation revealed that both dysbindin-1 and snapin are concentrated in synaptic tissue, including synaptic vesicle and postsynaptic density fractions. It is not detected in a presynaptic fraction lacking synaptic vesicles. Immunoelectron microscopy showed that dysbindin-1 is located in or on (1) synaptic vesicles in axospinous terminals of the dentate gyrus inner molecular layer and CA1 stratum radiatum and (2) postsynaptic densities and microtubules of dentate hilus neurons and CA1 pyramidal cells. The labeled synapses are often asymmetric with thick postsynaptic densities suggestive of glutamatergic synapses, most likely deriving from dentate mossy cells and CA3 pyramidal cells.

Discussion: The function of dysbindin-1 in presynaptic, postsynaptic, and microtubule locations may all be related to known functions of snapin.

114. Correlation of Prepulse Inhibition with Wisconsin Card Sorting Test Performance in Schizophrenia and Controls: Effects of Smoking Status

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Sponsor: Tony P. George

Background: Patients with schizophrenia exhibit deficits in prepulse inhibition (PPI) of the startle response, as well as high rates of cigarette smoking, and PPI has been found to be sensitive to cigarette smoking in schizophrenia. Further, this population is known to have deficits in executive functioning, as measured by neuropsychological tasks, including the Wisconsin Card Sorting Test (WCST). The baseline performance of patients with and without schizophrenia on PPI and WCST, and the effect of cigarette smoking on these measures has been established (George et al., 2006; Sacco et al., 2005). Further, there is a well-known linear relationship between PPI and WCST performance. We examined the relationship between PPI and WCST performance outcomes in schizophrenics and controls, and the effect of smoking status.
Methods: Pearson's product moment correlations were conducted between PPI and the major outcome measures of the WCST in four groups; smokers with schizophrenia (SS; n=12), nonsmokers with schizophrenia (SNS; n=7), nonpsychiatric control smokers (CS; n=13), and nonpsychiatric control nonsmokers (CNS; n=12).
Results: A significant correlation was found in the SS group between PPI 120 msec prepulse condition and the categories completed outcome of the WCST, the general measure of conceptual reasoning on this task ($r=0.64$; $p=0.024$). In contrast, no significant correlations between PPI and any WCST outcomes were observed in the SNS, CS, or CNS groups (all p's >0.20). Baseline differences amongst the four groups were observed for the PPI [$F=5.12$, $d=3,39$, $p<0.01$], with SNS demonstrating the poorest PPI, and SS demonstrated significantly better PPI than both SNS ($p=0.001$) and CS ($p=0.028$). Significant differences were also found amongst the four groups in terms of WCST Percentage Errors, % Perseverative Responses, % Perseverative Errors, and Categories Completed (all p's<0.05) with nonpsychiatric controls, irrespective of smoking status, outperforming schizophrenics on all outcome measures.
Discussion: Selected executive function outcomes of WCST (e.g. categories completed) are strongly associated with PPI in smokers with schizophrenia in comparison to non-smoking patients, and controls, suggesting that the association between sensorimotor gating and prefrontal executive functioning is enhanced by acute smoking. Our preliminary findings may contribute to understanding of the vulnerability of patients with schizophrenia to nicotine dependence, as well as targeted treatment of executive functioning and PPI deficits in this population. Supported in part by NIDA grants R01-DA-14039 and K02-DA-16611 (to TPG), and a 2005 NARSAD Young Investigator Award (to KAS).

115. Prefrontal Cortical Disruption of D2-GABA Modulation of Excitatory Synaptic Transmission in a Developmental Animal Model of Schizophrenia

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Sponsor: Travel Awardee, sanofi-aventis, 2006

Background: Prefrontal cortical (PFC) disruption in animals with a neonatal ventral hippocampal lesion (NVHL) has been proposed to model the cortical deficits observed in schizophrenia. In fact, mesocortical stimulation typically elicits a hyper-reactive response in the PFC of NVHL animals, an effect that could be observed only after puberty. This suggests that dopamine (DA) modulation of PFC neuronal activity is developmentally compromised in these animals.
Methods: To assess whether a NVHL affects D2 modulation in the PFC, we conducted whole-cell patch clamp recordings in brain slices from sham and lesioned animals at both pre- (PD<35) and post-pubertal (PD>60) ages. We investigated the effect of D2 receptors on deep-layers fast-spiking (FS) and non fast-spiking (NFS) interneurons excitability by comparing the number of spikes evoked by constant depolarizing current pulses before and after drug application. We also examined whether the temporal dynamics of D2 inhibition of excitatory synaptic transmission in deep-layers pyramidal neurons is disrupted in NVHL rats. A typical fast non-NMDA excitatory postsynaptic potential (EPSP) was obtained by electrical stimulation of layers I-II at a site about 1 mm lateral to the recorded cell.
Results: As observed in naïve rats (Tseng & O'Donnell, Cerebral Cortex 2006), bath application of the D2 agonist quinpirole (1-2 uM) significantly increased the excitability of FS (n=6) and NFS (n=5) interneurons by ~40 % in sham animals, but only at post-pubertal ages. This effect was not evident when recordings were conducted in presence of the D2 receptor antagonist eticlopride (20 uM, n=5) confirming that the effect is D2-dependent. In the lesioned group, quinpirole failed to increase the excitability of FS (n=6) and NFS (n=7) interneurons. Instead, the majority of NVHL interneurons (70 %) recorded remained unchanged after quinpirole, resembling the response observed in the PFC of pre-pubertal sham and NVHL rats. These results indicate that a NVHL prevent the acquisition of mature PFC interneurons response to DA. Next, we examined whether the D2 modulation of PFC excitatory synaptic transmission is affected by a NVHL. In the adult PFC, quinpirole reduced pyramidal neurons EPSP amplitude by ~20 % in both sham (n=6) and NVHL (n=7) animals, an effect that was completely blocked with eticlopride (n=7). However, the duration of this inhibition was significantly longer in sham animals as compared to the lesioned group. Pyramidal neurons recorded from NVHL rats recovered to baseline amplitude by ~10 min after quinpirole was removed, whereas a washout period of at least 25 min was required in the sham group. Interestingly, the duration of this long post-quinpirole effect in sham animals could be reduced to ~10 min with the GABA-A antagonist picrotoxin (10 uM, n=6). These suggest that part of the inhibitory action of D2 receptors on pyramidal neuron excitatory transmission is also compromised in the PFC of adult NVHL animals, particularly the one that involves activation of local GABAergic interneurons.
Discussion: In summary, D2 modulation of PFC inhibition and excitation become developmentally compromised in the PFC of NVHL animals. These changes could lead to the abnormal cognitive performance in NVHL animals by setting inappropriate coordination between pyramidal neurons and GABAergic interneurons, which in turn may alter the spatial selectivity of PFC neuronal response to excitatory inputs. A similar cortical disruption could be involved in schizophrenia, a disorder characterized by hypofrontality.

116. The Orexin-1 Antagonist SB-334867 Blocks Antipsychotic Treatment Emergent Catalepsy: Implications for the Treatment of Extrapyramidal Symptoms

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Background: We have previously shown that the orexin-1 antagonist SB-334867 blocks the electrophysiological effects of haloperidol and olanzapine on the activity of A9 and A10 dopamine neurons. These results indicate that orexin-1 antagonists might block some clinical effects of antipsychotic drugs. To evaluate if orexin-1 antagonists might potentially block other effects of antipsychotic drugs in animals, we examined the effects of SB-334867 on behavioral (catalepsy and exploratory locomotor

activity), neurochemical (elevation of dopamine metabolites), and neuroendocrine (elevation of serum prolactin) effects of antipsychotic drugs.
Methods: All experiments were performed in male, Sprague-Dawley rats. Catalepsy was measured in by using the bar test. Rats' front limbs were placed over a 2-cm high horizontal bar. The intensity of the catalepsy was measured by the time the rats remained in this position for a maximum of 120 sec. Exploratory locomotor activity was measured by placing rats immediately following antipsychotic injection into an automated locomotor activity cage and activity was measured for 60 min. For the measurement of prolactin and the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), rats were sacrificed 90 min following haloperidol administration and the n. accumbens and striatum were dissected and trunk blood was collected. DOPAC concentrations were measured using HPLC with electrochemical detection and prolactin was measured by using radioimmunoassay methods.
Results: Pretreatment with SB-334867 (0.01 – 10 mg/kg, IP) significantly decreased the catalepsy produced by the administration of haloperidol (1 mg/kg, SC), risperidone (2 mg/kg, SC), and olanzapine (10 mg/kg, SC). Administration of SB-334467 also reversed catalepsy after it had been established in animals pretreated two hours earlier with haloperidol. However, pretreatment with SB-334867 (1 – 10 mg/kg, IP) did not block the decreases in exploratory locomotor activity produced by administration of haloperidol (0.1 mg/kg, SC) or risperidone (0.3 mg/kg, SC). In addition, pretreatment with SB-334867 (1 – 10 mg/kg, IP) did not block the increased levels of DOPAC in the nucleus accumbens or striatum, nor the elevation in serum prolactin produced by administration of haloperidol (0.1 mg/kg, SC). Administration of SB-334867 alone did not change locomotor activity, DOPAC or prolactin levels, nor produce catalepsy.
Discussion: These results show that orexin-1 antagonists block the catoleptogenic effects of antipsychotics, but do not block other locomotor, neurochemical, or neuroendocrine effects of antipsychotics. Since catalepsy is thought to be a good predictor of extrapyramidal symptoms in humans, treatment with orexin-1 antagonists might decrease the occurrence or severity of antipsychotic treatment emergent extrapyramidal symptoms in humans.

117. Evidence of Nonsynonymous Polymorphisms of Neuregulin1 Gene Affecting Prepulse Inhibition Endophenotype in Schizophrenia

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Sponsor: Gunvant K. Thaker

Background: Neuregulin 1 (NRG1) is a leading schizophrenia candidate gene, with a locus mapped to chromosome 8p - a region believed to harbor one or more schizophrenia susceptibility genes. This gene was initially identified by its robust effect on rodent prepulse inhibition (PPI) (Stefansson et al 2002). PPI is one of the most widely adopted measures in animal models of psychosis, extensively used for evaluating antipsychotic drug action, and considered an endophenotype for schizophrenia. We hypothesized that if NRG1 is indeed conferring risk for schizophrenia, one should observe a similar genetic effect on PPI in a clinical population of schizophrenia patients. The genetic variants of NRG1 leading to the PPI or schizophrenia phenotypes are not established. So far 2 single nucleotide polymorphisms (SNPs) located at the exons of the gene have been identified as amino acid changing polymorphisms and are polymorphic with minor allele frequency greater than 5% (SNP1: rs3924999, a C>T polymorphism at the 2nd codon, which exchanges arginine to glutamine; and SNP2: rs10503929: T>C at 2nd codon exchanging methionine to threonine). In this study, we examined the potential neurophysiological effects of these two nonsynonymous SNPs on PPI using a case-control association study design.
Methods: Startling response was elicited using auditory startling sounds, while PPI was generated by a non-startling prepulse 120ms preceding the startling sound. PPI was measured as the percent suppression on startling eyeblink response following the prepulse. SNP typing was performed using TaqMan® SNP Genotyping Assays on an ultra-high throughput machine. We compared the genotypic effects of the two SNPs on PPI in 172 individuals, including subjects with schizophrenia (n = 96), first-degree family members of schizophrenia patients (n=30), and comparison participants without family history of schizophrenia (n=46).
Results: The distribution of genotypes was consistent with those predicted under Hardy Weinberg Equilibrium in all groups. Schizophrenia patients (%PPI: 39.4±27.1) had reduced PPI compared to controls (50.7±17.7) ($F(1, 107)=4.8$, $p=0.03$). Relatives (45.6±32.7) did not significantly differ from either group on PPI. There was no significant genotype effect on diagnosis for SNP1 (Chi-Squre=0.67, df=4, p=0.96) or SNP2 (Chi-Square=4.92, df=4, p=0.30). There was no significant genotype by diagnosis interactions in SNP1 (p=0.11) or SNP2 (p=0.80). There was a significant SNP1 genotype effect on PPI ($F(2,130)=4.03$, $p=0.02$). Percent PPI was lowest in homozygous minor allele mutation TT or glutamine/glutamine (31.5±28.0), intermediate in heterozygous CT (40.4±31.5), and highest in homozygous major alleles CC or arginine/arginine (49.0±20.9). The same effects were observed in schizophrenia patients alone (p=0.05) and in healthy controls alone (p=0.03), but not in the relatives group (p=0.71). One additive model suggested that SNP1 alone contributes to 7% of the PPI variance. There was no significant SNP2 genotype effect on PPI (p=0.99).
Discussion: We conclude that this mis-sense mutation (rs3924999) on the neuregulin 1 gene may have a functional effect on prepulse inhibition.

118. Dehydroepiandrosterone (DHEA) for Persistently Symptomatic Schizophrenia Patients

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Background: The glutamatergic hypofunction hypothesis of schizophrenia accounts for many aspects of disease pathogenesis. However, available antipsychotic treatments do not offer direct activity at glutamate receptors, and impaired glutamate neurotransmission may contribute to the lack of efficacy of dopamine antagonists in many persistently symptomatic patients. Dehydroepiandrosterone (DHEA) is a naturally secreted and abundant neurosteroid that is known to enhance NMDA receptor mediated neurotransmission, possibly through agonist activity at the sigma receptor. One recent study found that physiological doses of DHEA augmentation improved negative and affective symptoms in schizophrenia (Strous et al., 2003). In the current study, the effect of supraphysiological doses of DHEA in treatment-refractory schizophrenia was investigated, with the hypothesis that DHEA augmentation would ameliorate multiple dimensions of psychopathology.
Methods: 30 male subjects with schizophrenia or schizoaffective disorder that had persistent symptoms (min. PANSS score = 60 [61-127]) with prior adequate trials of typical and atypical antipsychotics and maintained for at least 4 weeks on a stable dose of an atypical antipsychotic were randomized to adjunctive DHEA (titrated up to 400 mg/day by week 4) or placebo for 6 weeks. PANSS total score was the primary outcome measure analyzed by ANOVA using LOCF for 27 patients (15 DHEA, 12 placebo) that completed at least 2 weeks of treatment.
Results: PANSS total change scores between baseline and 6 weeks did not differ between subjects who received DHEA (7.13 + 2.43, mean + sd) vs placebo (2.25 + 2.85, p=0.202). Likewise, no differences emerged between the groups on the positive subscale (p=0.118), the negative subscale (p=0.734), general psychopathology (p=0.446),

CGI (p=0.520), and AIMS (p=0.937), although there was a trend for Simpson-Angus (p=0.077).
Discussion: This study did not demonstrate efficacy for supraphysiological doses of DHEA in persistently symptomatic patients with schizophrenia. Although the numerical change scores for clinical and side-effect rating scales were all in the direction of greater improvement for DHEA, none met statistical significance at $p<0.05$. It is noteworthy that a recent study reported improvement in extrapyramidal side-effects (EPS) in DHEA augmented patients, given the trend towards an amelioration of EPS in the current study. Factors that may have contributed to the negative results – given the one earlier positive study – include the possibility that DHEA is only effective in more treatment-responsive patients and in those selected specifically for prominent negative symptoms, and that any beneficial effects are relatively subtle and would require a larger sample size to define. Funding for this study provided by the Stanley Medical Research Institute.

119. Expression of the NMDA Receptor Trafficking Proteins CASK, Velis, Mint1 and KIF17 in Frontal Cortex in Schizophrenia

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Sponsor: James H. Meador-Woodruff

Background: In recent years, clinical observations of glutamate involvement in aspects of the pathophysiology for schizophrenia have been supported by numerous studies of altered cortical and subcortical expression of the glutamate receptor complex in postmortem brain. Evidence suggests that, in addition to regional changes of expression of the NMDA receptor and its interacting PSD proteins in schizophrenia, cellular processing including dendritic trafficking might also be compromised in this illness. A cellular mechanism for microtubule trafficking in the dendrite of NMDA receptors has recently been described. Following assembly of the NMDA receptor, a protein complex consisting of the mLin7/Velis (1-3) and mLin10/Mint1 adaptor proteins associated with the Shank related mLin2/CASK anchoring protein, link vesicles containing newly synthesized NMDA receptors to the microtubule associated kinesin related KIF17 motor protein. NR2B-containing NMDA receptors in particular rely on this protein complex for dendritic targeting as cellular knockdown of KIF17 selectively affects trafficking of NR2B containing NMDA receptors, whereas NR2A containing receptors that rely on different trafficking mechanisms are unaffected. Altered expression of the KIF17-associated NMDA receptor trafficking complex in schizophrenia would cause an altered composition of NMDA receptor subtypes at the PSD, and due to differences in channel properties cause altered NMDA mediated signaling at the PSD without necessarily causing a change in the total number of receptors. To study the hypothesis of compromised dendritic trafficking in schizophrenia, we have in this study measured expression of the Velis, Mint1, CASK, KIF17 molecular complex on transcript and protein levels in two prefrontal cortical areas known be associated with abnormal NMDA mediated signaling in schizophrenia.
Methods: Postmortem brain from patients with schizophrenia and a comparison group were obtained from the Mount Sinai Medical Center Brain Bank. For in-situ hybridization, clones for each transcript (CASK, Velis, Mint1 and KIF17) were generated using a PCR amplified 200-400 bp cloned fragment specific for each transcript. [S35] labeled antisense mRNA, synthesized from each clone, was used to detect specific transcript expression in cryostat sectioned brain tissue as we have previously described. For protein detection, brain homogenates from each subject were run in duplicate (30µg/lane) on 7.5% (CASK, KIF17, Mint1) or 15% (Velis) precast gels (Bio-Rad) and subsequently transblotted onto PVDF membranes. Detection of specific protein expression was done using commercially available antibodies for each protein by western blot. Quantification of transcript and protein expression was done as previously described.
Results: At present, we have analyzed expression of Mint1 and Velis3 in dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) in brains from patients with schizophrenia and a comparison group. Mint1 transcript expression in DLPFC was significantly increased in schizophrenia whereas Velis3 was not altered. Expression of Mint1 and Velis3 in ACC was not altered. Currently, we are in the process of analyzing expression of the remainder of these trafficking molecules at transcript and protein levels in both DLPFC and ACC.
Discussion: In this study, we have measured expression of proteins associated with a microtubule associated trafficking complex responsible for dendritic trafficking of the NMDA receptor in DLPFC and ACC in patients with schizophrenia and comparison subjects. Increased Mint1 transcript expression in schizophrenia suggest that altered NMDA receptor trafficking, in addition to alterations in expression of the receptor and associated PSD proteins, might be involved in the pathophysiology of schizophrenia.

120. Gambling Task Performance in Schizophrenia and Nicotine Dependence

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Background: The Iowa Gambling Task (IGT) assesses risk-reward decision-making. Some but not all studies have found that individuals with schizophrenia (SZ) perform disadvantageously on the task whereas more consistent deficits in SZ have been elicited with the Wisconsin Card Sorting Task (WCST), a test of executive function. Prior studies in non-SZ groups found differences in IGT performance related to tobacco smoking. However, the relationship between tobacco smoking and IGT performance has not been systematically examined in individuals with psychotic disorders.
Methods: Neurocognitive assessment of SZ smokers (n=32) and non-smokers (n=13) and non-SZ (CON) smokers (n=15) and non-smokers (n=12) was performed. Smokers were assessed under non-deprivation conditions.
Results: The SZ group trended towards worse performance (selection of fewer cards from advantageous decks) on the IGT (SZ vs. CON scores: 4.7 vs. 16.8; p=0.07). Non-significant within-diagnostic-group differences were observed in opposite directions in relationship to tobacco use status (SZ, smoker vs. non: 5.8 vs. 2.0, $p>0.1$; CON, smoker vs non: 12.9 vs. 21.7; $p>0.1$). Across all groups, correlations between IGT and WCST performance ($r=0.30$ to $r=0.33$, p's<0.01) were observed on multiple WCST measures (errors, perserveration, and category completion), and, within the SZ group, correlations reached significance at $p<0.05$ only for perseverative responses and errors (for each, $r=0.31$).
Discussion: Across SZ and CON smoking and non-smoking groups, worse IGT performance correlates with worse WCST performance suggesting an overlap in cognitive processes assessed by the IGT and WCST persistent across diagnostic groups. Among SZ subjects, the significant correlations between IGT and WCST performance on perseverative measures suggests that difficulties in shifting patterns of behaviors might be particularly salient for decision-making in SZ. Although individuals with SZ performed marginally worse than CON subjects on the IGT, preliminary data suggest that these differences are not significantly modified by smoking status. This study was supported in part by NIDA (MNP, TPG), VA MIRECC (MNP) and NARSAD (KAS, TPG).

121. Category Processing in Schizophrenia

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Sponsor: Martha Shenton

Background: In this study we examine categorization processes in schizophrenia using Event Related Potential (ERP) methodology. There is a general recognition that the ability to sort objects and

events into categories is fundamental to shaping human cognition and the questions about processes of categorization are questions about the structure of human memory (see Keri, 2003 for a review). Few ERP studies explored categorical processing (e.g., Kiefer, 2005; Batty and Taylor, 2002; Ellis and Nelson, 1999; Mouchetant-Rostaing et al., 2000 and Folstein and van Petten, 2004). These authors identified a no-go N200 and the frontal late positive component (LPC) as sensitive to processing category information. The no-go N200 has been associated with inhibitory processes and conflict monitoring (e.g., Schmitt et al., 2000, 2001) and frontal LPC with multi-dimensional categorization processes (Folstein and Van Petten, 2004). Few behavioral studies explored categorization processing in schizophrenia (e.g., Green, 2004; Elvevag, 2004) and there are no ERP studies of category processing in SZ.
Methods: Five male schizophrenia patients (SZ) and 6 normal control subjects (NCs) categorized abstract creatures as either 'Mogs' or "Nibs' according to a learnt rule. The creatures either had all three features representing the category (far boundary exemplars- FBE) or two features (near boundary exemplars - NBE). There were three parts to the study: the rule acquisition, the training, and the testing part in which ERP and accuracy data were recorded. The EEG was recorded in a 64 electrode montage.
Results: Following training, there was a high accuracy rate in both SZ (FBE:95.9%; NBE:75.8%) and NCs (FBE:100%; NBE: 91%). ERPs were characterized by a no-go N200 and the frontal LPC. For both FBE and NBE more positive no-go N2 was found in SZ ($p< 0.045$) relative to NC; and less positive frontal LPC was found in SZ only to FBE ($p<0.05$) relative to NC. In examining within group differences, the far and near boundary categories in the NCs differed in the frontal LPC in keeping with Folstein and van Petten findings (2004). In contrast in SZ the grand averages to far and near boundary stimuli were nearly identical.
Discussion: These findings suggest that both initial processes of feature selection and inhibition of irrelevant features and later processes of accessing memory and comparing a set of features against a rule-based prototype are abnormal in schizophrenia. This is the first ERP study that we are aware of to report such effects.

122. Efficacy and Safety of Bifeprunox versus Placebo in the Treatment of Patients with Acute Exacerbations of Schizophrenia

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Sponsor: Russell Poland

Background: To determine whether 30 mg or 40 mg bifeprunox treatment is superior to placebo in patients with acute exacerbations of schizophrenia, and to evaluate the safety and tolerability of bifeprunox as compared to placebo.
Methods: A 6-week randomized, double-blind, placebo-controlled, risperidone-referenced safety and efficacy study of bifeprunox included 599 randomized subjects with acutely exacerbated schizophrenia (DSM-IV-TR). Patients were randomly assigned to bifeprunox 30 mg (n=148), bifeprunox 40 mg (n=148), placebo (n=149) or risperidone 6 mg (n=154). Bifeprunox doses were titrated, beginning with a dose of 0.25 mg on day 1 and approximately doubled every day until 30 mg or 40 mg (day 8) were reached, while risperidone was titrated over 3 days. The change in the Positive and Negative Symptom Scale (PANSS) total score (baseline to endpoint) was the primary outcome measure (LOCF). Secondary efficacy measures included: Clinical Global Impressions (CGI)-Severity of Illness, PANSS negative, PANSS positive, PANSS-derived Brief Psychiatric Rating Scale (BPRS) score, BPRS psychosis cluster, and CGI-Improvement scores. Safety and tolerability evaluations included extrapyramidal symptoms (EPS), weight gain, lipid profile, and serum prolactin.
Results: Compared to placebo, bifeprunox 30 mg produced a statistically significant (P=0.020, 95% CI = -10.3 to -1.4) improvement in the PANSS total score at 6-week endpoint, and was associated with notable improvement in PANSS positive and general psychopathology subscale, BPRS total score, BPRS psychosis cluster score and CGI-I responder rate. Bifeprunox 40 mg significantly separated from placebo on PANSS positive symptom subscale (P=0.020, 95% CI = -3.2 to -0.3) and BPRS psychosis cluster score (P=0.031, 95% CI = -1.9 to -0.1). Discontinuation due to adverse events (AEs) was comparable across all treatment groups. Bifeprunox 30 mg was associated with decreased levels of prolactin at endpoint compared with placebo, while risperidone treatment increased prolactin levels. Bifeprunox and placebo had similar incidence (30 mg bifeprunox: 6%; 40 mg bifeprunox: 8%; placebo: 5%) of EPS while the risperidone group had a greater incidence (14%) of EPS versus placebo. The use of anticholinergic medication was similar between bifeprunox and placebo. The most common AEs for patients on bifeprunox (incidence >5% and twice for placebo) included: nausea, vomiting, constipation, dyspepsia, diarrhea, dizziness, and decreased appetite. In addition, bifeprunox produced statistically significant ($P<0.005$) decreases in weight compared to placebo and risperidone at endpoint. Bifeprunox reduced non-fasting glucose at week 3 and endpoint, and decreased triglyceride levels. It was not associated with clinically significant increases in QTc interval.
Discussion: In this study, bifeprunox 30 mg was effective and safe. A decrease in body weight and improvement in the lipid profile was noted in patients treated with bifeprunox. The efficacy, safety and tolerability profile of bifeprunox suggest it may be a novel and effective agent to employ as a treatment option for patients with schizophrenia.

123. Risk Factors for Schizophrenia: Are They Specific for Mental Illness or for Undesirable Events in General?

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Sponsor: Michael Davidson

Background: Previous studies have identified various risk factors for schizophrenia, including low cognitive and social abilities, having a non-psychotic psychiatric diagnosis in adolescence (anxiety, depression, personality disorders), and fewer years of formal education. Some but not other studies indicated that cigarette smoking and low socio-economic status (SES) also increase risk. Many of these studies indicate that these risk factors have relatively low sensitivity or specificity. We examined the specificity of these risk factors by assessing their effect on risk for going to jail during military service, as a proxy for.an undesirable life event.
Methods: We identified 627,103 male adolescents for whom data were available from the assessments performed by the Israeli draft board, and examined the effect of these putative risk factors for schizophrenia on risk for going to jail for 7 days or more during their military service. Those adolescents diagnosed with psychotic disorder during draft board assessment or later hospitalized with any psychotic disorder (N=3,839) were excluded, leaving 623,264 adolescents in the analysis. All potential risk factors were dichotomized to low (more than 1 SD below population mean or higher), and normal-high (1 SD below population means or higher).
Results: Low social functioning (OR=1.14), low cognitive functioning (OR=3.48), low SES (OR=2.09), less education (OR=6.26), having a non-psychotic psychiatric diagnosis (anxiety, depression, personality disorder, OR=2.01) and cigarette smoking (OR=3.55) were associated with going to jail.
Discussion: Some of the risk factors associated with schizophrenia are associated with risk for undesirable life events such as going to jail during military service. One might hypothesize that in persons with these risk factors, the presence or absence of other environmental

and/or genetic risk factors causes mental illness to manifest in some, while others have undesirable life events, such as going to jail, without suffering from psychosis.

124. Differential Effects of Quetiapine, Olanzapine and Risperidone on Glucose Metabolism in Patients with Schizophrenia: Results from a 24-Week, Randomized Study

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Sponsor: Stephen Zukin

Background: This study compared effects of quetiapine, olanzapine or risperidone on glucose metabolism in non-diabetic patients with schizophrenia.
Methods: This multicenter, randomized, 24-week, open-label, flexible-dose, parallel-group study (D1441C00125) used primary endpoint of baseline to Week 24 change in AUC 0-2h plasma glucose during an oral glucose tolerance test (OGTT), a sensitive measure of glucose regulation; primary analysis compared quetiapine and olanzapine. Patients were hospitalized overnight to ensure 8-14h fasting conditions before OGTT. Secondary analyses included change in fasting (0 min) and 2-h glucose, insulin parameters (fasting levels, AUC 0-2h plasma insulin following OGTT, index of insulin sensitivity [ISI] and HOMA), HbA1c, weight, fasting lipids; statistical significance was analysed post hoc based on CIs. Key laboratory values were blinded.
Results: 395 patients (quetiapine 115, olanzapine 146, risperidone 134) had OGTT data at baseline and ≥20 weeks' treatment (mean mg/day: quetiapine, 607; olanzapine, 15.2; risperidone, 5.2). A significant (p=0.048) difference was observed between quetiapine and olanzapine in the primary endpoint, change from baseline in AUC 0-2h plasma glucose (mg/dL×h), with significant increases during treatment with olanzapine (+21.9, 95% CI 11.5, 32.4) and risperidone (+18.8, CI 8.1, 29.4), but not quetiapine (+9.1, CI -2.3, 20.5). There were no statistically significant between-treatment differences in weight, insulin parameters, fasting glucose or HbA1c. Following OGTT, AUC 0-2h plasma insulin statistically significantly increased from baseline with olanzapine (24.45%, CI 11.46, 38.96) but not quetiapine (13.15%, CI -0.14, 28.22) or risperidone (10.74%, CI -1.2, 24.13). Change from baseline in ISI was statistically significant with olanzapine (-19.1%, CI -27.9, -9.33) and risperidone (-15.8%, CI -25.1, -5.41) but not quetiapine (-10.8%, CI -21.9, 1.85). Small increases in mean fasting glucose and HbA1c within the normal range occurred in all groups (statistically significant for quetiapine and risperidone). Fasting glucose changes: quetiapine 3.18 mg/dL (CI 0.24, 6.12); risperidone 4.40 (CI 1.62, 7.18); olanzapine 2.33 (CI -0.40, 5.06). HbA1c changes: quetiapine 0.122% (CI 0.054, 0.191); risperidone 0.065% (CI 0.001, 0.129); olanzapine 0.05% (CI -0.01, 0.112). LDL-C increased statistically significantly with quetiapine (13.3 mg/dL, CI 6.1, 20.5) and olanzapine (20.5, CI 13.8, 27.1) but not risperidone (5.1, CI -1.8, 11.9) (olanzapine vs risperidone statistically significantly different). Post hoc analysis indicated triglyceride/HDL and total cholesterol/HDL ratios increased statistically significantly from baseline with olanzapine but not quetiapine or risperidone.
Discussion: Significant reductions in glucose tolerance, the primary endpoint, were observed with olanzapine and risperidone but not quetiapine, with a statistically significant difference between olanzapine and quetiapine.

125. 1H Magnetic Resonance Spectroscopy in Non-Psychotic Young Relatives at Risk for Schizophrenia

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Background: Proton magnetic resonance spectroscopy (1H MRS) suggests neurochemical abnormalities such as reduced N-acetylaspartate (NAA) as well as alterations in the glutamatergic function in schizophrenia. The question of whether these abnormalities reflect trait related vs. state related alterations in schizophrenia is not clear. We examined MRS alterations in young relatives of schizophrenia patients (High Risk subjects, HR) using proton MRS. We hypothesized that HR subjects, especially those with already evident psychopathology, will have alterations in neurochemical integrity in corticostriatal and thalamic brain structures.
Methods: We obtained short echo-time, multi-voxel 1H MRS scans using a 1.5T GE scanner in 27 young consenting relatives [offspring] at risk for schizophrenia (HR), and 37 age and gender matched healthy controls (HC) at the University of Pittsburgh Medical Center. Axis I non-psychotic disorders were seen in 10 HR subjects (PHR) but no psychopathology was seen in 17 HR subjects (NHR). Absolute metabolite levels of NAA, phosphocreatine plus creatine (Pcr+Cr), glycerophosphocholine plus phosphocholine (GPC+PC), myo-inositol (Ins) and Glutamate (Glu) were assessed in 7 different right and left regions (prefrontal white matter, anterior cingulate (ACC), basal ganglia, temporoparietal/occipital, thalamus and posterior white matter regions) using LC Model. Multivariate analyses using age, gender and side as covariates were conducted to look at group effects on regional metabolite measures.
Results: Significant NAA (p=0.0068), PCr+Cr (p=0.045) and glutamate (p=0.019) reductions were seen in HR subjects in the basal ganglia, as well as a significant Ins increase in the ACC (p=0.029), PCr+Cr (p=0.020) and glutamate (p=0.020) reductions in the temporoparietal region, and glutamate reductions in the thalamus (p=0.023) all compared to HC subjects. Post-hoc analyses revealed more pronounced NAA, PCr+Cr and glutamate reductions in the temporoparietal regions in PHR relative to control subjects (p<0.0002). NAA, glutamate, Ins and PCr/Cr levels were reduced in basal ganglia in the NHR subjects relative to the PHR and HC subjects (p<0.03).
Discussion: Our findings suggest abnormalities in the neurochemical integrity of the corticostriatal regions in young relatives at risk for schizophrenia. The observed glutamate reductions may reflect either alterations in glutamatergic neurotransmission, consistent with the NMDA hypofunction theory of schizophrenia or a nonspecific reduction in the metabolic glutamate pool.

126. The Nicotinic Alpha7 Receptor Agonist MEM 3454 Increases Dopamine and Acetylcholine Release in Rat Medial Prefrontal Cortex and Hippocampus Alone and in Combination with Risperidone

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Sponsor: Herbert Y. Meltzer

Background: The cholinergic system plays a major role in attention, learning, and memory, which is due, in part, to its ability to modulate the release of neurotransmitters such as dopamine (DA) which have a known role in cognition. Previous microdialysis studies in rats have provided conflicting evidence as to whether agonists at the alpha4beta2 nicotinic acetylcholine receptor (nAChR) or the alpha7 nAChR stimulate hippocampal (HIP) or mesoprefrontal cortical (mPFC) DA and acetylcholine (ACh) release in rodents.
Methods: We have now utilized microdialysis in awake freely moving rats to study the effects of MEM 3454, an alpha7 nAChR partial agonist with 5-HT3 receptor antagonist properties on these measures.
Results: MEM 3454 (0.3, 0.45 and 0.6 mg/kg, s.c) significantly increased DA and ACh efflux in both the mPFC and HIP. At a dose of 1.0 mg/kg, MEM 3454 induced only a slight increase in HIP ACh efflux. Both low and high dose MEM 3454 (0.1 and 10 mg/kg) failed to modulate either DA or ACh efflux. MEM 3454 (0.45, 1.0 and 3.0

mg/kg, s.c.) did not affect DA and ACh efflux in the nucleus accumbens (NAc). Pre-treatment with the selective alpha7 nAChR antagonist methyllycaconitine (MLA, 1.0 mg/kg, s.c.) produced a significant and complete blockade of DA, but not ACh, efflux induced by MEM 3454 (0.45 mg/kg) in both the mPFC and HIP. Administration of a low subthreshold dose of MEM 3454 (0.1mg/kg) in combination with the atypical antipsychotic risperidone (0.1 mg/kg, s.c.) failed to potentiate the effects of DA and ACh efflux above the levels induced by the antipsychotic when given alone. Administration of a higher dose of MEM 3454 (0.45 mg/kg, s.c.) with risperidone (0.1 mg/kg), however, significantly increased DA and ACh efflux in both brain regions. The combined effect of MEM 3454 (0.45 mg/kg) and risperidone on DA efflux in the mPFC and HIP appeared to be additive, whereas the effects on ACh efflux appeared to both additive and supra-additive in the mPFC and HIP, respectively.
Discussion: These results provide a possible mechanism by which MEM 3454, alone or combined with risperidone, might improve cognitive dysfunction in schizophrenia by increasing DA and ACh release in cortex and hippocampus. Moreover, this study indicates that alpha7 nAChR stimulation contributes to the MEM 3454-induced DA efflux in rat mPFC and HIP.

127. Psychosocial Functioning in Pediatric Patients Treated with Open-Label Ziprasidone

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Background: We set out to examine the baseline psychosocial functioning in children and adolescents with psychotic disorders and to evaluate outcomes following treatment with open-label ziprasidone.
Methods: Subjects (aged 10–17 years) were randomized to open-label ziprasidone monotherapy titrated over 7 to 10 days from 10 to 40 mg bid (Group 1) or from 20 to 80 mg bid (Group 2). Treatment at fixed doses continued for up to 3 weeks (Period 1), after which subjects could continue flexible-dose treatment for 6 months (Period 2), with concomitant therapy permitted. Study inclusion criteria were diagnosed bipolar I disorder (manic or mixed) with a Young Mania Rating Scale (YMRS) score ≥17, or diagnosed schizophrenia/schizoaffective disorder with a Brief Psychiatric Rating Scale–Anchored (BPRS-A) score ≥4 on at least 1 of the items for unusual thought content, hallucinations, suspiciousness, or conceptual disorganization. The Child Health Questionnaire (CHQ; completed at baseline) and the Children's Global Assessment Scale (CGAS; completed on Day 4 and at Weeks 1, 2, 3, 4, 12, and 27) were employed to assess psychosocial functioning. Subjects were stratified by age into 3 groups for CHQ assessments: 11–12 years, 13–15 years, and 16–18 years.
Results: In total, 23 subjects were enrolled into Group 1 (15 bipolar, 8 schizophrenic/schizoaffective) and 40 subjects into Group 2 (31 bipolar, 9 schizophrenic/schizoaffective). Because the CHQ was administered only at baseline, the results are summarized across both dose groups. More than 90% of subjects scored below normal for their age on the Family Activities, Impact on Parent Time, Impact on Parent Emotions, Mental Health, Self-Esteem, and Behavior subscales of the CHQ. Subjects aged 13–15 years and 16–18 years were at or above peer norms (60% and 61.9% of subjects, respectively) for the Physical Health Global Summary subscale, whereas 60% of children aged 11–12 years scored below the median. Baseline mean CGAS scores (SD) were 41.74 (9.87) for Group 1 and 38.98 (10.00) for Group 2; no subject scored ≥70. Mean increases from baseline to Week 3 were 14.41 (13.65) for Group 1 and 17.41 (15.44) for Group 2; large gains were observed by Week 1 and were maintained for up to 6 months. By the end of treatment Week 3, 5 subjects scored ≥70. Effect size for the CGAS across both groups was 1.35 (95% CI, 0.97–1.78). Among subjects with bipolar I disorder, treatment responders (i.e., ≥30% decrease in YMRS scores by the end of Period 1) had a mean increase in CGAS of 22.28 (SD, 14.11), whereas nonresponders had a mean increase of 4.21 (SD, 13.38) ($P<0.0001$). Among subjects with schizophrenia/schizoaffective disorder, treatment responders (i.e., ≥30% decrease in BPRS scores by the end of Period 1) had a mean increase in CGAS of 17.67 (SD, 9.75), whereas nonresponders had a mean increase of only 3.27 (SD, 5.02) ($P<0.0001$).
Discussion: During 3 weeks of treatment with ziprasidone, both dosing groups showed gains in functioning that paralleled symptom improvement, as demonstrated by the efficacy measures. Most of the improvement in psychosocial functioning occurred early in the course of treatment. Thus, the CHQ and CGAS may be useful to detect deficits in psychosocial functioning in these populations. Additionally, this study demonstrates that the CGAS is sensitive to change with antipsychotic treatment. Children and adolescents with bipolar I disorder or schizophrenia/schizoaffective disorder who are treated with ziprasidone exhibit clinically meaningful improvement in psychosocial functioning as early as the first week of treatment, with sustained improvements for up to 6 months.

128. Similar Rates of Agitation, Anxiety and Insomnia in Sedating and Non-Sedating Antipsychotics: Evaluating Clinical Trial Results with Aripiprazole, Haloperidol and Olanzapine

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Sponsor: Martin Reite

Background: Calming rather than sedation has been identified as the goal in managing agitation. However, calming and sedation have not been well described and their relationship is obscure. Aripiprazole is an atypical antipsychotic with a unique mode of action and a receptor signature substantially different from other antipsychotic drugs. Although it has demonstrated calming in agitated patients, patients initiating treatment with aripiprazole may experience symptoms such as agitation, anxiety and insomnia, particularly when switching from full D2 antagonists with significant sedative and anticholinergic properties.
Methods: To quantify and qualify agitation, anxiety and insomnia, an analysis was undertaken in schizophrenia patients receiving aripiprazole (Ari), haloperidol, (Hal) or olanzapine (Olz).
Results: At endpoint (26 or 52 weeks), Ari- and Hal-treated patients reported comparable rates of anxiety (Ari: 11.8%; Hal: 10.4%), agitation (Ari: 5.7%; Hal: 7.2%) and insomnia (Ari: 20.5%; Hal: 18.8%). Similarly, under double-blind conditions, Ari and Olz patients reported comparable rates of anxiety (Ari: 14.3%; Olz: 13.7%), agitation (Ari: 6.4%; Olz: 8.3%) and insomnia (Ari: 24.8%; Olz: 20%). The majority of patients (≥75%) reported these AEs during the first 12 weeks of the long term trials. Among Hal-treated patients, agitation onset was noted in a median of 3 days and insomnia and anxiety onset somewhat later (median of 12 and 16 days, respectively). In the Ari group versus Hal, the median onset for agitation, anxiety and insomnia was 14, 12, and 9 days, respectively. Similarly, in the Olz group, agitation onset was earlier at 5 days whereas insomnia and anxiety occurred later (median of 10 and 12 days, respectively). For the Ari group versus Olz, the median day of onset for anxiety and insomnia was 10 days and for agitation 11 days. In the Hal comparative trial, the median duration of the AEs was of 1-2 weeks and similar in Ari and Hal groups. In the Olz comparative trials, the median duration of these AEs was much shorter (≤ 3 days) for both Ari and Olz groups. For all three AEs, most cases (≥80%) were reported as mild or moderate and rarely led to discontinuation in all groups. BZD use was similar for all groups, varying from 55 to 61% among patients not reporting AEs and from 87 to 91% for those with anxiety, agitation and insomnia.
Discussion: Under double-blind conditions, some aspects of anxiety, agitation and insomnia appear similar for aripiprazole and haloperidol, two non-sedating antipsychotics. More interesting, these AEs

were also similar for aripiprazole and olanzapine, contrary to assumptions about sedative effects of olanzapine. High BZD use may have masked or altered the course and severity but should not do so differentially. Taken together, these results suggest that agitation, anxiety and insomnia are not directly related to sedation; however, more data are warranted to support this preliminary conclusion.

129. The Partial NMDA Antagonist Memantine Normalizes Brain Activity in Frontal and Parietal Regions: A Controlled fMRI Study

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Sponsor: Henry A. Nasrallah

Background: Research over the past decade suggests that cognitive impairments are a significant component of the symptomatology and neurobiology of schizophrenia. These cognitive impairments are associated with functional deterioration during the course of schizophrenia and are better predictors of functional outcome than other clinical dimensions of the illness. Working memory deficits have been identified in medicated, unmedicated, chronic, and first-episode schizophrenic patients. We hypothesized that in schizophrenic patients treatment with memantine, a noncompetitive NMDA receptor antagonist that has been shown to improve cognition in patients with Alzheimer's disease, would improve cognitive function beyond the effects of atypical antipsychotics alone and would normalize functional brain activation.
Methods: To investigate our hypothesis, we performed fMRI evaluations in a subset of schizophrenic patients who were enrolled in a placebo-controlled double-blind clinical trial of memantine (MEM-MD-29). Seven residually symptomatic patients with DSM-IV schizophrenia on stable atypical antipsychotic treatment (olanzapine or risperidone) were recruited along with seven age and gender matched healthy comparison subjects. All subjects performed a series of n-back tasks (0-, 1-, 2-, and 3-back) during the scanning session. The n-back task is a well-established parametric measure of working memory that is widely used in studies of psychiatric patients. While the 0-back condition represents a simple measure of attention and is the control condition, the 1- 2- and 3-back conditions are tests of working memory that parametrically increase memory load, and, consequently, task difficulty. During the MEM-MD-29 trial, fMRI scans were obtained from schizophrenic subjects at baseline (during the 1-week screening period), after 1 week of memantine or placebo adjunctive treatment, and at the completion of the 8-week study. Control subjects had only a single fMRI scan.
Results: Behavioral measures included reaction time and accuracy. There were no significant differences ($p>.05$) in either measure between healthy subjects and schizophrenic patients treated with memantine or placebo, nor were there differences between the two schizophrenic groups. Both healthy and schizophrenic subjects showed significantly increased brain activation ($p<.01$) in left inferior frontal gyrus and decreased activation in the left inferior parietal lobe with increasing memory load, determined by subtracting activation in the 2-back task from the 1-back task. Within the schizophrenic subjects, treatment with memantine compared to placebo caused increased activation in the left inferior frontal gyrus and decreased activation in the left inferior parietal lobule ($p<.01$).
Discussion: Previous neuroimaging studies in healthy controls have shown the involvement of the inferior frontal gyrus and left inferior parietal lobe in working memory. However, prior studies of schizophrenic patients found divergent patterns of activation in these two regions compared to healthy controls. Therefore the current results suggest schizophrenic subjects taking memantine have brain activation that shifts closer to the profile of healthy controls in these two regions, suggesting memantine may normalize brain activation in schizophrenic patients. The implications of these findings will be discussed in light of the glutamate hypothesis of schizophrenia.

130. Modafinil Effects on Prefrontal-Dependent Cognition in Schizophrenia: fMRI and Electrophysiology Studies

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Cognitive dysfunction is a critical feature of schizophrenia, and remains largely resistant to treatment with existing antipsychotic medications. Modafinil is an FDA-approved medication with a novel profile of neurochemical effects, including elevation of extracellular dopamine, norepinephrine and glutamate in the frontal cortex. The results of preliminary behavioral cognition studies suggest that it may be beneficial for cognition in schizophrenia. We hypothesize that modafanil's neuropharmacological profile may favor the remediation of cognitive deficits in schizophrenia by supporting local network function in the prefrontal cortex. We compared the effects of modafinil on measures of brain activity in clinically-stable schizophrenia patients and in healthy adults during performance of a task with context and conflict processing demands.
Methods: To date, five schizophrenia patients and nine healthy adults have been enrolled. Each underwent a single-dose, double-blind placebo-controlled test of oral Modafinil 200 milligrams on two separate days within a one-week interval. On each day, between 2-4 hours post-dose all subjects participated in separate fMRI and ERP sessions during the performance of a cognitive task (Preparing to Overcome Prepotency) which contains context- and conflict-processing demands.
Results: Current results indicate a trend toward a significant main effect of drug condition (modafinil > placebo) on accuracy on the POP task. BOLD response in fMRI, and both Gamma-range oscillatory activity and Error-Related Negativity magnitude in the ERP paradigm, are currently being analyzed.
Discussion: Full analyses will be presented, with increased sample sizes to test the hypothesis that modafinil treatment is associated with improvement of prefrontal-dependent cognitive deficits in schizophrenia.

131. Long-Term Efficacy and Safety of Bifeprunox in Patients with Schizophrenia: A 6-Month, Placebo-Controlled Study

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Sponsor: Rachel Klein

Background: Objectives: To determine the long-term efficacy of bifeprunox in patients with stable schizophrenia by assessing the time to deterioration with bifeprunox, compared to placebo, over a period of up to 6 months. In addition, the study investigated whether clinical effect as assessed by the PANSS scale, after 6 weeks of treatment with bifeprunox, is superior to placebo. The safety of bifeprunox will also be examined.
Methods: A 6 month randomized, double-blind, placebo-controlled, parallel-group, multi-center study of bifeprunox included 497 randomized patients with a diagnosis of schizophrenia (DSM-IV) and stable symptomatology. Patients were randomly assigned to receive a once-daily, fixed dose of bifeprunox 20 mg, bifeprunox 30 mg or placebo. Bifeprunox doses were titrated, beginning with a dose of 0.25 mg on day 1 and approximately doubled every day until 20 mg (day 7) or 30 mg (day 8) were reached. The primary outcome measure was time to deterioration following randomization (LOCF). Time to deterioration from randomization was defined as fulfillment of

one or more of the following criteria: CGI-Improvement (CGI-I) score ≥5, or PANSS item P7 (hostility) and /or G8 (uncooperativeness) score ≥5 for 2 consecutive days, or ≥20% increase in PANSS total score from baseline. The key secondary measure was change from baseline in PANSS total score at week 6. Additional secondary measures included: PANSS positive, negative, and general psychopathology, PANSS-derived Brief Psychiatric Rating Scale (BPRS) score, BPRS psychosis cluster, Clinical Global Impressions-Severity of Illness (CGI-S), and CGI-I scores. Safety and tolerability evaluations included extrapyramidal symptoms (EPS), weight/BMI, metabolic parameters, serum prolactin and ECG recordings.
Results: Treatment with bifeprunox resulted in significantly longer time to deterioration of schizophrenia (bifeprunox 20 mg: P=0.008 and bifeprunox 30 mg: P=0.006) compared to placebo. The proportion of patients who deteriorated at endpoint was 59% in the placebo group, 41% in the bifeprunox 20 mg group, and 38% in the bifeprunox 30 mg group. Bifeprunox also showed a statistically significant improvement for PANSS total score at Week 6. In addition, statistically significant improvement was seen for PANSS total scores, PANSS positive and general psychopathology subscale scores, BRPS total scores, and BPRS psychosis cluster scores compared to placebo, at most timepoints. The most common adverse events (incidence >5% and twice for placebo) included: nausea, vomiting, decreased appetite, dizziness and akathisia. Prolactin levels decreased in all treatment groups. Patients receiving bifeprunox demonstrated an improved lipid profile, with triglycerides decreasing and mean HDL values increasing. Weight decrease was also seen in patients receiving bifeprunox, with statistical significance ($P<0.05$) compared to placebo in the bifeprunox 30 mg group at endpoint.
Discussion: In this study, it was demonstrated that doses of 20 and 30 mg/day were effective in preventing deterioration over 6 months in patients with stable schizophrenia. Bifeprunox may be beneficial in maintaining long-term stability in stable patients with schizophrenia. A favorable metabolic profile was seen for bifeprunox, based on weight changes, lipids and the presence or absence of metabolic syndrome.

132. Altered Phosphorylation of Ionotropic Glutamate Receptors in the Dorsolateral Prefrontal Cortex in Schizophrenia

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Sponsor: Margit Burmeister

Background: Glutamate receptor subunits are multiply phosphorylated by several protein kinases. Phosphorylation regulates several functional properties of these receptors, including ion conductance, endoplasmic reticulum (ER) exit, dendritic trafficking, membrane targeting, turnover and degradation. Thus, protein phosphorylation is an essential feature in synaptic function, especially in synaptic plasticity, where glutamate receptor activation results in signaling cascades that lead to activation of transcription factors and modulation of gene expression. It has been suggested that phosphorylation of NMDA and AMPA subunits by multiple kinases, including PKA, PKC, and CaMKII, regulates receptor ion channel function and location. Each stage of trafficking (endocytosis/storage/exocytosis-lateral translocation) for both receptor families is regulated by phosphorylation of the receptor subunits themselves, or of the chaperone proteins with which they interact in the postsynaptic density. In particular, in-vitro studies have shown that phosphorylation of NR1 at Ser896 and Ser 897 regulates NMDA receptor exit from the ER and insertion at the membrane, and phosphorylation of Ser831 and 845 in GluR1 regulates the ion channel properties and synaptic trafficking of GluR1-containing AMPA receptors during hippocampal LTP.
Methods: Postmortem samples of the dorsolateral prefrontal cortex from schizophrenic and control brains from the Mount Sinai Brain Bank were studied. After homogenizing the tissue, we analyzed protein levels of NR1 and GluR1 subunits by western blot analysis, detecting in the same samples the expression of the phosphorylated forms of both subunits: NR1pSer896 and pSer897, and GluR1pSer831 and pSer845.
Results: We found increased expression of NR1pSer896 but normal levels of NR1pSer897. Decreased expression of GluR1pSer845 and normal levels of GluR1pSer831 where also detected in the same subjects.
Discussion: These data indicate that phosphorylation states for select molecules are preserved in postmortem tissue and that it is feasible to detect alterations in receptor subunit phosphorylation in this illness. Phosphorylation of NR1Ser896 and GluR1Ser845 residues modulates receptor exit from the ER, thus these data suggest abnormal ER transit of the NMDA and AMPA receptor complexes in schizophrenia. Dephosphorylation at GluR1Ser845 is associated with the endocytosis of AMPA receptors at the postsynaptic density. Interestingly, NR1pSer897 and GluR1pSer831, phosphorylation of which are associated with receptor sensitivity and affinity for glutamate, were not changed. These results suggest abnormal distribution of the subcellular location of the glutamate receptors containing these subunits, reflecting a reduction in the availability of glutamate receptors to be inserted in the membrane and diminished glutamate signal transduction in the dorsolateral prefrontal cortex in schizophrenia.

133. Effect of Divalproex on 5-HT-2a Receptor Function in Healthy Human Subjects

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Background: Preliminary data from previous studies suggests that divalproex treatment may have significant effects on 5-HT synaptic function/transmission in human subjects (Maes et al., 1997; Shah et al., 1997). Collectively, these studies suggest that divalproex may increase 5-HT release and that 5-HT receptors may downregulate in response. This study was designed to determine if treatment with divalproex would affect CORT response to m-CPP Challenge (i.e., 5-HT-2a receptor mediated response) in healthy male volunteers.
Methods: Twelve healthy male subjects underwent two sets of m-CPP/Placebo Challenges studies before and after at least three weeks of treatment with divalproex.
Results: Placebo-Corrected Delta CORT Responses demonstrated no difference between pre-treatment and post-treatment time points. However, plasma m-CPP levels were significantly lower at the post-treatment time point ($p < .001$) by about 30%.
Discussion: These data demonstrate no apparent effect of divalproex treatment on 5-HT-2a receptor responsiveness in healthy human subjects. However, a reduction in the bioavailability of the m-CPP Challenge agent after divalproex treatment suggests the possibility that equivalent CORT responses to m-CPP may actually represent increased 5-HT-2a mediated responsiveness after treatment with divalproex.

134. Tagging and Capture in Synaptic Plasticity

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Background: Long-lasting synaptic plasticity is believed to support the persistence of memory traces in the brain. Frequently studied models for such plasticity are long-term potentiation (LTP, produced by high frequency stimulation, HFS) and long-term depression (LTD, produced by low-frequency stimulation, LFS) at the CA3-CA1 synapse in hippocampus. Weak HFS and LFS (wHFS, wLFS) produce transient potentiation and depression, respectively, named early LTP (E-LTP) and early LTD (E-LTD). Strong stimulations (sHFS, sLFS) produce very long-lasting changes: late LTP (L-LTP) and late LTD (L-LTD). These forms of LTP require protein synthesis in the stimulated

neurons, which, to a large extent, occurs in the dendrites, using dendritic mRNA. The work of Frey and Morris (Nature 385, 533; 1997) has shed additional light on the process. These authors have shown that when a weak stimulus in one pathway is coupled to a strong stimulus in another, it too can cause an L-LTP rather than an E-LTP. The experiments are done in rat hippocampus slices, synaptic potentials are measured by extracellular electrodes, and two separate pathways to the same neurons are stimulated. When a weak stimulus in one pathway follows a strong stimulus in the other by as much as 75 minutes, the weak stimulus will now produce an L-LTP rather than an E-LTP. This was recently formalized by Tonegawa and colleagues (Kelleher et al. Neuron 42, 773; 2004), as follows: the weak stimulus marks synapses by a creating a tag, consisting of a protein modified chemically, e.g. by phosphorylation. The strongly stimulated synapses synthesize plasticity related proteins (PRP's) that are then captured by the tag, allowing the weakly stimulated synapses to elaborate a full blown L-LTP

Methods: The experiments were done in rat hippocampus slices, synaptic potentials were measured by extracellular electrodes, and two separate pathways to the same neurons were stimulated. The stimuli could be either strong or weak, at a low frequency or a high frequency and separated by as many as 75 minutes (strong before weak)

Results: The present experiments were designed to test a prediction of Tonegawa's model, namely that if a protein synthesis blocker (e.g. anisomycin) is applied to the preparation after the strong stimulus but before the weak one, capture should still occur, because the proteins about to be captured would have already been synthesized by the earlier, strong stimulus. We find that this prediction is not fulfilled, using two different stimulus combinations (sHFS/wHFS, sLFS/wHFS). In each case the application of 20 μM of anisomycin, applied before the weak stimulus and continuing for the duration of the recording, completely prevented capture, yielding an E-LTP in response to the weak stimulus. L-LTP induced in the first pathway remained intact throughout the anisomycin exposure.

Discussion: Our data thus support the model of Blitzer et al. (Biol.Psych. 57,113; 2005) whereby the synapses subjected to weak stimulation, following a strong stimulation in a converging pathway, synthesize their own plasicity related proteins (PRP's), rather than capture them from neighboring, strongly stimulated synapses. The tag would thus not be required to capture PRP's, but rather make specific mRNA available for their translation. The strongly stimulated synapses will help the weakly stimulated ones to synthesize PRP's by exporting increased translational capacity,e.g. activating the vital mTOR translational pathway. Indeed, application of the mTOR inhibitor rapamycin also prevented capture. Supported by NIDA grant DA015863 to E.M.L.

135. Structural Analysis of the Antidepressant- and Cocaine-Sensitive Serotonin Transporter Using a Homology Model-Driven Approach

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Background: The serotonin transporter (SERT) is responsible for removing the neurotransmitter serotonin from the synapse following vesicular release. This action is a primary mechanism for termination of receptor-mediated signaling in the serotonergic system. Many drugs including the antidepressants, cocaine, and the amphetamines act on the serotonin transporter. Because the transporter is a clinically important drug target, there is much interest in gaining a better understanding of the structure of SERT as it relates to the molecular aspects of drug binding.

Methods: Although the crystal structure of SERT has yet to be resolved, the structure of a related bacterial leucine transporter has been reported. A homology model of SERT has been generated from the coordinates of the *Aquifex aeolicus* leucine transporter ($LeuT_{Aa}$) structure using the hSERT sequence and energy minimization. This model has provided the opportunity to generate new testable hypotheses regarding SERT structure and ligand recognition. Using a combination of site-directed mutagenesis, cysteine scanning mutagenesis, and computational modeling of ligand binding, we have tested our homology model seeking to validate the model and gain insight into SERT function.

Results: Two complementary studies have been performed. The first set of studies was designed to verify the homology model by mapping the entrance to the putative permeation pathway. Using the substituted cysteine accessibility method (SCAM), we have generated several cysteine mutants in transmembrane helices (TMHs) I, VI, X, XI as well as extracellular loop IV. The residues chosen for mutagenesis are predicted to encircle the entrance to the substrate permeation pore. Reactivity with methanethiosulfonate (MTS) compounds as well as bifunctional MTS cross-linking reagents has confirmed the proximity of these residues to each other and to the transporter permeation pathway. A second set of studies has focused on identifying regions of the transporter involved with ligand recognition. Mutagenesis studies in TMHs II, III, VII, and XII as well as extracellular loops IV and V have been performed in conjunction with a Comparative Molecular Field Analysis (CoMFA) of the SERT ligand binding pocket.

Discussion: Our results suggest that several of these domains may directly contribute to the binding site of antidepressants and psychostimulants such as cocaine, whereas TMH VII specifically plays a role in sodium binding and substrate recognition. The diverse functions of TMH VII are made possible by a predicted conformational change in this domain that occurs as part of the translocation process. These data confirm the validity of our SERT homology model, as they are consistent with the predicted functions of the residues examined. Having a homology model of SERT and other biogenic amine transporters offers unparalleled opportunities to explore transporter function. Such homology models based on the $LeuT_{Aa}$ structure establish a new standard for comparisons for all structure/function studies of Na^+-dependent transporters. These studies supported by NIH grant R01DA018682-05.

136. 5-HT4 Receptor, a GPCR Implicated in Memory, Activates MAP Kinase Through a Pathway Independent of G-Proteins and B-Arrestin but Dependent of Src Kinase

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Sponsor: Jonathan Javitch

Background: Among the G-protein coupled receptor (GPCR) genes, the 5-HT4R gene is one of the largest (700kb) . All the 5-HT4R splice variants are expressed extensively in the brain and at the periphery. They are implicated in important physiological functions such as memory, cognition, feeding, respiratory control, and gastro-intestinal motility. The 5-HT4Rs are involved in all these physiological functions mainly by activating the Gs/cAMP pathway. In mice colliculi neurons the 5-HT4R-mediated increase in cAMP levels after a short application of agonist inhibited voltage–gated K+ channels, as well as including Ca2+ -activated K+ channels and their closure. These activities are mediated by cAMP and PKA activation. cAMP produced, via the activation of 5-HT4Rs, can also increase the activated exchange factor Epac involved in the stimulation of soluble amyloid precursor protein alpha (sAPPA) secretion. A recent report shows that cAMP-dependent PKA is involved in the pathway, inducing MAP kinase, however the mechanism of MAPK activation through Gs-coupled receptors is not fully understood. 5-HT4Rs appear to use multiple pathways to activate the MAPK cascade and seem to depend on different factors.

Methods: Experiments were carried on primary cultures of mouse colliculi neurons expressing endogenously 5-HT4R and on HEK 293 cell lines transiently expressing 5-HT4R WT or mutants. Western blots or cell surface ELISA or measurement of second messengers (cAMP or inositol phosphates) were performed on both systhems. ERK1/2 Phosphorylation and Src phosphorylation assays were performed by analysing the cell lysates on blots with antibodies to pERK1/2, to pSrc (Tyr 418) and to total Src (Tyr 527). Protein-protein interactions were revealed by Immunofluorescence and confocal microscopy or by co-immunoprecipitation analyzed on western blots.
Results: We show that activation of 5-HT4R receptor stimulates rapidly and transiently (2–5-min) the Erk1/2. Using different mutants with different coupling led us to reveal that this receptor known to be preferentially coupled to Gas in neuronal cells but also to Gaq in transfected cell lines did not used theses pathways to stimulate the MAPK kinase. The independence towards Gs-protein signaling and cAMP is supported by the absence of effect of PKA inhibitor H89, or forskolin. In contrast, our data demonstrate that Src is a major component of 5-HT4R-stimulated MAPK42/44 activation. However, b-arrestin, one of the major GPCR scaffolding protein, is not required for the activation of the MAPK by 5-HT4R. Src strongly associates constitutively to 5-HT4R, but Src phosphorylation and activation depends on 5-HT4R stimulation. This particular signaling also occurs in colliculi neurons where 5-HT4R is endogenously expressed.
Discussion: MAPK activation could be important to understand the processes implicated in 5-HT4R-dependent memory reinforcement. Indeed previous data has demonstrated that 5-HT4R positively modulate learning and memory and that the MAPK cascade is critically involved in the mechanisms underlying modification of neuronal networks required for the stability of memories. All these results emphazise the high level of interest in this signaling molecule and on new 5-HT4R signaling pathways.

137. Extinction Reverses the Conditioning-Induced Depression of Infralimbic Neuronal Excitability

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Sponsor: Travel Awardee, NIMH, 2006

Background: Neurons in the infralimbic (IL) subregion of the medial prefrontal cortex (mPFC) show potentiated tone-evoked responses during recall of extinction (Milad and Quirk, 2002), yet the cellular mechanisms of this change are not understood. To test if fear extinction enhances the intrinsic excitability of IL neurons, we combined behavior and brain slice electrophysiology.
Methods: Three experimental groups were examined: 1) rats that received fear conditioning on day 1 and extinction on day 2 (EXT group); 2) rats that received conditioning on day 1 but no extinction on day 2 (No-EXT group) and 3) rats that remained in their home cages throughout (Naïve group). On day 3, freezing levels to test tones were 22%, 70% and 1% for EXT, No-EXT and Naïve groups, respectively, indicating good recall of extinction in the EXT-group. Immediately after this test, coronal slices were prepared and the intrinsic excitability of IL pyramidal neurons was assessed by whole cell patch-clamp recordings. A total of 111 IL cells (EXT: 44, No-EXT: 36, Naïve: 31) were identified as pyramidal neurons based on their morphology.
Results: Depolarizing steps evoked significantly more spikes in the EXT group than the No-EXT group ($p < 0.01$). However, the EXT group did not differ from the Naïve group, indicating that conditioning depressed IL excitability and that this was reversed by extinction. We also observed that the hyperpolarization-activated cation current (Ih) was significantly reduced in the No-EXT group compared to the other two groups ($p < 0.01$).
Discussion: Enhancing Ih conductance might be a mechanism by which extinction returns IL excitability to pre-conditioning levels. Given that IL inhibits amygdala output (Quirk et al., 2003), decreased IL excitability could relieve a tonic brake on fear, thereby facilitating the expression of fear responses (Garcia et al., 1999).

138. Which HPA-Axis Alterations Are Specific for PTSD? Assessment of Early Awakening Cortisol Response, Response to Low Dose Dexamethasone and to DEX/CRH Challenge in a Sample of PTSD Patients, Trauma and Healthy Controls

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Sponsor: H.M. van Praag

Background: While enhanced cortisol suppression in response to dexamethasone is one of the most consistent biological findings in patients with posttraumatic stress disorder (PTSD), the relative contributions of psychopathology and trauma exposure to this finding remains unclear. This also holds true for other alterations in HPA-axis regulation in PTSD.
Methods: We assessed the early morning cortisol response, cortisol response to to 0.5 mg dexamethasone, and assessed cortisol in the combined DEX/CRH test in a sample of veterans with PTSD (n=23), veterans without PTSD (trauma controls, TC) (n=22), and healthy controls (HC) (n=24). In addition we assessed 4 pm plasma cortisol, adrenocorticotrophic hormone (ACTH) and corticotrophin binding globulin (CBG) in response to dexamethasone in PTSD patients and trauma controls. All subjects had been assessed with SCID and CAPS; the PTSD and TC sample was matched for age, year and region of deployment. The HC sample was age matched. Five patients were medication free for at least 4 weeks, all other patients were naïve for psychotropic medication. All tests and assessments were timed in order to prevent spilling of effects.
Results: Both PTSD patients and TC subjects demonstrated significantly more salivary cortisol suppression compared to HC subjects. Salivary cortisol, plasma cortisol and ACTH suppression as well as CBG levels did not differ between PTSD and TC subjects. In both PTSD and TCs a reduced awakening cortisol response (0-30 min) was found in comparison to HCs. No significant differences were observed in ACTH and cortisol response in the DEX-CRH challenge between PTSD and TC. PTSD patients with comorbid MDD showed a significantly lower ACTH response to CRH compared to patients without comorbid MDD. The response to the DEX-CRH challenge did not correlate with either PTSD or depressive symptoms.
Discussion: In our groups enhanced cortisol suppression to 0.5 mg dexamethasone was related to trauma exposure and not specifically to PTSD. This was further supported by a reduced awakening cortisol response in both PTSD patients and trauma controls. Patients with PTSD did not respond differently to a DEX-CRH challenge than traumatized controls. However, the presence of a comorbid MDD attenuated the ACTH response. These results provide new information on the specificity of HPA-axis alterations for PTSD.

139. The Neuroinflammatory Response and Oxidative Stress Induced by Acute Intracerebroventricular Administration of Lipopolysaccharide Are Decreased in Cyclooxygenase-1 Deficient Mice and Increased in Cyclooxygenase-2 Deficient Mice

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Sponsor: Past Travel Awardee, Bristol Myers Squibb, 2003

Background: Neuroinflammation is a key component in the progression of some neurodegenerative disorders, including Alzheimer's disease. Epidemiological data support a beneficial effect of cyclooxygenase (COX) inhibition in preventing or delaying the onset of Alzheimer's disease. However, clinical trials using mostly COX-2 selective inhibitors in patients with mild to severe symptoms of

Alzheimer's disease have been unsuccessful. COX enzymes represent the rate-limiting step in the metabolism of arachidonic acid to prostaglandins (PGs), a pathway activated in neuroinflammation. However, the specific role of each of COX-1 and COX-2 isoforms in these processes is still unclear. Elucidating the role of individual COX in neuroinflammation would help to develop better therapeutic approaches for those neurodegenerative diseases with a marked inflammatory component. We investigated the role of COX-1 and COX-2 in the neuroinflammatory response induced by intracerebroventricular injection of lipopolysaccharide (LPS) using COX-1 deficient (COX-$1^{-/-}$), COX-$2^{-/-}$ and wild type mice.

Methods: The study was approved by the NIH Animal Care and Use Committee in accordance with NIH guidelines on the care and use of laboratory animals. Saline or LPS dissolved in saline (5μg/5μl) was injected into the lateral ventricle in 3 month-old mice anesthetized with ketamine/xylazine and placed in a stereotaxic apparatus. Gene expression was measured by real time PCR, protein analysis by Western blotting and immunohistochemistry. PG levels were measured by ELISA in lipid extracts from brains subjected to high-energy microwave irradiation to stop metabolism.

Results: In wild type mice LPS increased activated microglia in the cortex, corpus callosum, and cerebral ventricles and resulted in morphological changes of astrocytes in the cortex, hippocampus, and areas surrounding the cerebral ventricles. These changes were accompanied by the up-regulation of the proinflammatory mediators IL-1β, TNFα and PGE_2. Reactive gliosis, expression of proinflammatory cytokines, and brain PGE_2 levels, and translocation and activation of nuclear factor-κB and mitogen-activated protein kinases, important factors for signaling events during an inflammatory response, were significantly decreased in COX-$1^{-/-}$ mice. Protein oxidation, a critical factor contributing to the secondary progression of the inflammatory reaction and oxidative damage was also reduced in the COX-$1^{-/-}$ mice. In contrast, the mRNA expression of IL-1β, TNFα, and of markers of activated microglia and astrocytes (CD11B and GFAP) was significantly increased after LPS injection in the COX-$2^{-/-}$ mice compared to wild type mice.

Discussion: These observations suggest that COX-1 enhances whereas COX-2 attenuates LPS-induced neuroinflammatory response and imply that COX-1 inhibitors rather than COX-2 selective inhibitors should be considered for the treatment of neurodegenerative diseases with a marked inflammatory component.

140. Fluphenazine-Induced Hypokinesia in C3H/HeN Mice is Attenuated by Melatonin (MLT) Through Activation of MT1 MLT Receptors

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Sponsor: Minority Faculty Fellow

Background: Extrapyramidal symptoms (EPS) are prominent side effects of antipsychotic drugs [Casey, CNS Spectr.11(Suppl 7):25-31, 2006], and are generally treated by lowering the drug dose or with anti-cholinergic agents. We previously reported that acute pharmacological treatment with MLT ameliorated the EPS hypokinesia induced by the typical antipsychotic, fluphenazine, in rats (Sumaya et al., Pharmacol Biochem Behav. 78:727, 2004) and in C57BL/6 mice (Sumaya et al., Soc. Neurosci. 29, Abstr. 512.4, 2003, Online) known to be negligible producers of MLT. The goal of this study was to assess the role of endogenous MLT and its receptors on fluphenazine-induced hypokinesia in C3H/HeN mice with genetic deletion of the MLT receptors. This mouse strain, as in humans, expresses a robust circadian rhythm of pineal MLT (Masana et al., J. Pineal Res. 28:185, 2000).

Methods: The effect of exogenous MLT on fluphenazine-induced hypokinesia was studied in male C3H/HeN mice (14/10h L/D cycle; ZT=0 lights on; Zeitgeber Time: ZT) with a targeted deletion of either the MT1, MT2 or MT1/MT2 (double knock out) MLT receptor(s). Hypokinesia was induced by pretreatment with the D2 antagonist, fluphenazine (1mg/kg, i.p.), 2.5 hrs prior to a bartest (ZT9.5-10.5) which was followed by treatment with either vehicle (VEH) or MLT(10mg/kg, s.c.) 30 min prior to testing. The effects of VEH or the competitive MLT receptor antagonist, luzindole (10mg/kg, s.c.), at two time points (15 min before fluphenazine and 30 min before the bartest) was tested in C3H MT2KO mice pretreated with fluphenazine (1mg/kg, i.p.) 2.5 hrs prior to a bartest (ZT9.5-10.5). A maximum of 300 sec was allowed on the bartest.

Results: C3H wild type (WT) and MT2 knockout mice (MT2KO) displayed similar robust levels of hypokinesia after treatment with fluphenazine (WT:193.5±26.6 sec, n=8; MT2KO:169.5±42.2 sec, n=9). In both WT and MT2KO mice, MLT treatment dramatically reduced fluphenazine-induced hypokinesia by 95% (WT:10.4±2.1 sec, n=9, $p<0.001$) and 86% (MT2KO:23.0± 5.4 sec, n=9; $p<0.01$), respectively, as compared to VEH treated. Fluphenazine treatment, however, did not induce the expected hypokinesia in either MT1KO or MT1/MT2 KO mice (MT1KO:25.4±3.0 sec, n=9; MT1/MT2 KO:26.4±7.5 sec, n=9). MLT treatment did not affect the negligible levels of hypokinesia in either genotype (MT1KO:17.0±4.0 sec, n=8; MT1/MT2 KO:18.2±5.6 sec, n=8). To further understand the role of the MT1 MLT receptor in fluphenazine-induced hypokinesia, we tested the effect of luzindole, on fluphenazine-induced hypokinesia in mice with a targeted deletion of the MT2 MLT receptor. The luzindole treated group showed a potentiation of fluphenazine-induced hypokinesia as compared to the VEH treated group (VEH:105.6±20.8 sec, n=12; luzindole:240.5±20.9 sec, n=12; $p<0.001$).

Discussion: These data suggest that the ability of the D2 antagonist, fluphenazine, to induce hypokinesia in the C3H MLT producing mouse as well as the ameliorating effect of MLT is due, at least in part, to the presence of MT1 MLT receptors. We conclude that, in rodents with a similar diurnal pattern of MLT secretion as found in humans, the attenuating effect of MLT on fluphenazine-induced hypokinesia is probably mediated through activation of the MT1 MLT receptor. The potentiation of fluphenazine-induced hypokinesia by luzindole treatment in mice lacking the MT2 receptor suggests blockade of an MT1 MLT receptor activated by endogenous MLT. These findings provide support for the possible use of MLT as an adjunct treatment to ameliorate extrapyramidal side-effects induced by antipsychotic treatment. Supported by MH 42922(MLD)

141. The Effects of Transcranial Magnetic Stimulation over the Left Prefrontal Cortex on Pain Perception in Healthy Adults, Gastric-Bypass Surgery Patients and Patients with Chronic Neuropathic Pain

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Sponsor: Mark S. George

Background: Several studies have found that rTMS delivered over motor cortex can affect the perception of laboratory-induced pain in healthy adults as well as chronic neuropathic pain in clinical samples. While the motor cortex has been a popular target for pain management, there is evidence that TMS over the prefrontal cortex can cause secondary activation of pain and mood-regulating regions, like the cingulate gyrus, orbitofrontal cortex, insula, and hippocampus. There is evidence to support the concept that left prefrontal activation is negatively correlated with pain experience suggesting a governing role of the prefrontal cortex on pain perception potentially via μ-opioid, norepinephrine and serotonergic systems. More research is needed to substantiate the left-prefrontal cortex as a potential cortical target for altering pain perception in healthy adults, post-operative and chronic neuropathic pain populations.

Methods: Study-1: Twenty healthy adults with no history of depression or chronic pain underwent thermal pain threshold assessment

using the method of limits with a TSA-II Neurosensory Analyzer before and after 15-minutes of either real or sham left-prefrontal rTMS (10Hz, 100%rMT, 2 secs ON, 58 secs OFF; 300 pulses). Study-2: Twenty patients undergoing gastric-bypass surgery were assigned to receive 20-minutes of real or sham rTMS (10Hz, 100%rMT, 10 secs ON, 20 secs OFF, 4000 pulses) in the Post-Anesthesia Care Unit immediately following surgery. Patient Controlled Analgesia (PCA) usage was monitored for the duration of each patient's hospital stay. Additionally, subjects used visual analogue scales twice per day to rate pain and mood. Results from this 20-subject replication study were combined with results from the initial trial (total N=40) to provide more accurate effect-size estimates. Study-3: Four patients with chronic neuropathic pain participated in a within-subject cross-over study of the effects of 3-days of real versus sham rTMS treatment (10Hz, 100%rMT, 10 secs ON, 20 secs OFF; 3-days; 12,000 pulses). MRI-images along with Rogue Research's Brainsite Frameless Stereotaxi system were used to locate the left-prefrontal cortex for each subject. Pain diaries were used to capture daily pain ratings. Subjects also underwent thermal and mechanical pain threshold assessment procedures in the laboratory.

Results: Study-1: Subjects that received active TMS evidenced a significant increase in thermal pain threshold following TMS, however thermal pain thresholds of subjects that received sham TMS did not change significantly. Study-2: Patients that received active TMS used 37% less PCA morphine at discharge than subjects that received sham. The slopes of the cumulative morphine usage curves differed significantly between groups. When data from both trials was combined (N=40), significant differences emerge between groups with respect to average morphine usage at discharge and ratings of post-surgical "pain at its worst" but not for "pain on average", or "mood". Study-3: Three days of active rTMS was associated with a decrease in average daily pain ratings compared to sham TMS lasting approximately 12 days after the rTMS treatment. Additionally, active TMS was associated with increases in thermal and mechanical pain thresholds.

Discussion: The left prefrontal cortex is a promising cortical target for altering pain perception in healthy adults, post-operative, and chronic neuropathic pain patients. Future studies are needed to begin to elucidate possible neurochemical mechanisms of action.

142. Molecular Genetics of the Platelet Serotonin System in First-Degree Relatives of Patients with Autistic Disorder

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Sponsor: Edwin Cook

Background: Schain and Freedman first described elevated whole blood serotonin (5-hydroxytryptamine, 5-HT) in autistic disorder more than four decades ago. Approximately one-third of individuals with autistic disorder are found to have elevated whole blood 5-HT levels. Genetic variation in the serotonin pathway might delineate a subgroup of patients with autistic disorder and shed light on the genetic liability to the syndrome.

Methods: Indices of the platelet serotonin system, including whole blood serotonin (5-HT), [14C]-5-HT binding affinity for the serotonin transporter (Km), [14C]-5-HT uptake (Vmax), and [3H]-LSD-labeled receptor binding were previously studied in twelve hyperserotonemic and twelve normoserotonemic first-degree relatives of probands with autistic disorder. These subjects were then genotyped for polymorphisms in the SLC6A4, HTR7, HTR2A, TPH1, and ITGB3 genes.

Results: Previous studies in an indepedent population yielded an a priori prediction of SLC6A4 haplotypes that separated the subjects into three groups with significantly different [14C]-5-HT binding affinity (Km, $p = 0.005$) and [14C]-5-HT uptake rate (Vmax, $p = 0.046$). Genotypes at the four individual polymorphisms in SLC6A4 were not associated with platelet serotonin indices. Haplotypes at SLC6A4 and individual genotypes of polymorphisms at SLC6A4, HTR7, HTR2A, TPH1, and ITGB3 showed no significant association with whole blood 5-HT. Haplotype analysis of two polymorphisms in TPH1 revealed nominally significant association with whole blood serotonin ($p = 0.046$).

Discussion: With the exception of the SLC6A4 haplotype association with [14C]-5-HT binding affinity, these exploratory association analyses of several SNPs with indices of the serotonin system are not robust to correction for multiple testing but generate hypotheses for confirmation in other samples.

143. Reliability of Low Frequency Reaction Time Oscillations in Adult Controls and Preliminary Data in Patients with Psychiatric and Addictive Disorders

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Background: Biological systems exhibit periodic fluctuations which are particularly well known in cardiology and neuroscience. In 1876 Mayer described 6-12 second oscillations in heart rate and blood pressure. "Mayer waves", are "low frequency oscillations" (LFO), characterized by spontaneity (they occur without apparent overt stimuli), slowness (frequency < 0.1 Hz which distinguishes from cardiac and respiratory cycles) and modulability (they are susceptible to being altered by pharmacological challenge). LFO are robustly observable in physiological recordings but typically filtered out. Ruskin, Walters and colleagues (2001) described LFO in the firing rates of neurons in the rat basal ganglia which were exquisitely sensitive to drugs that modulate dopaminergic (DA) transmission. We are intrigued by the possibility that LFO may be associated with "default mode" brain functional networks observed during rest (Raichle et al., 2001; Fransson, 2005). Default mode activity is linked to trial-by-trial variability and its suppression is associated with better cognitive performance in healthy subjects. Children with attention deficit hyperactivity disorder (ADHD) exhibit high amplitude LFO in reaction time (RT) time-series that are attenuated by methylphenidate (Castellanos et al (2005).

Methods: The goals of these ongoing studies are to characterize LFO in adults using an Eriksen flanker choice-reaction-time task and to begin to explore LFO in healthy normals and in patients with neurological and psychiatric conditions which are associated with DA dysfunction or which are treated with medications affecting DA neurotransmission. Over 80 adults provided written informed consent and completed at least one 180-trial Eriksen flanker test session. Many subjects completed multiple sessions to permit quantification of test-retest reliability, and exploratory analyses on the effects of fatigue, medication or other state variables. Reaction time (RT) data were subjected to fast Fourier transform and to Morlet wavelet analyses.

Results: Test-retest data from over 30 adult controls showed a high degree of within-subject reproducibility (ICC > 0.65, $p<.001$), for mean RT, RT SD, and LFO amplitude. Across adult populations mean RT, RT SD and LFO amplitude increased with age. Greater variance in RT SD and in LFO amplitude were seen broadly across patient populations with ~40% of patients falling above the range encompassing ~90% of the non-patient controls.

Discussion: Quantitative analysis of reaction time time-series may operationalize an endophenotype linking attentional and cognitive deficits to spontaneous fluctuations of underlying brain functional networks. This quantitative phenotype has adequate test-retest reliability in adult controls, and may have applicability across diverse psychiatric and addictive disorders.

144. Treatment of Pathological Gambling with a Glutamate-Modulating Agent, N-Acetyl Cysteine

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Background: Although pathological gambling is relatively common, investigation of response to pharmacotherapy in individuals with pathological gambling is limited. N-acetyl cysteine (NAC), an amino acid and cysteine pro-drug, appears to improve glutamatergic tone within the nucleus accumbens and therefore offers promise in mediating reward-seeking behavior.
Methods: 27 subjects (12 women) with DSM-IV PG were treated in an 8-week open-label trial of N-acetyl cysteine (NAC) with responders (defined as a ≥ 30% reduction in Yale Brown Obsessive Compulsive Scale Modified for Pathological Gambling [PG-YBOCS] total score at endpoint) randomized to 6 additional weeks of double-blind NAC or placebo.
Results: PG-YBOCS scores decreased from a mean of 20.3 ± 4.1 at baseline to 11.8 ± 9.8 at the end of the open-label phase ($p<0.001$). Sixteen of 27 subjects (59.3%) met the responder criteria. The mean effective dose of NAC was 1476.9 ± 311.3 mg/day. Of the 16 responders, 13 entered the double-blind phase. Of those assigned to NAC, 83.3% still met responder criteria at the end of the double-blind phase, compared to only 28.6% of those assigned to placebo.
Discussion: Treatment with NAC appears to be a safe and effective treatment for pathological gambling. The efficacy of NAC lends support to the hypothesis that pharmacological manipulation of the glutamate system may target core symptoms of reward-seeking addictive behaviors. NAC, a derivative of the naturally occurring amino acid cysteine, may be beneficial in treating impulse control disorders due to its hypothesized mechanism of modulating glutamate within the nucleus accumbens. Preclinical studies have suggested that levels of glutamate within the core of the nucleus accumbens mediate behavioral counterparts of animal cravings. Larger, longer, placebo-controlled, double-blind studies are warranted.

145. Prevalence of Impulse Control Disorders in Adolescent Psychiatric Inpatients

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Background: Data suggest that impulse control disorders are relatively common, carry significant morbidity and mortality, and may be effectively treated with behavioral and pharmacological therapies The age of onset for impulse control disorders appears to be in adolescence, but research concerning how common these disorders are in adolescents is currently lacking. As empirically validated treatments for impulse control disorders are available, it is important for psychiatrists to identify and treat people with these disorders. We aimed to examine the frequency of co-occurring impulse control disorders in adolescents who were voluntarily hospitalized in a psychiatric inpatient facility.
Methods: 102 consecutive inpatient psychiatric admissions [56 (54.9%) females; mean age = 15.8 ± 1.4 (range 13-18)]were screened for impulse control disorders using the Minnesota Impulsive Disorders Interview, a semistructured clinical interview assessing pathological gambling, trichotillomania, kleptomania, pyromania, intermittent explosive disorder, compulsive buying, compulsive sexual behavior, and pathological skin picking. Subjects screening positive for an impulse control disorder were then evaluated with a structured clinical interview by a psychiatrist with expertise in impulse control disorder who was unaware of the particular impulse control disorder(s) for which a subject screened positive. The percentages of patients with current and lifetime impulse control disorders, and 95% confidence intervals, were determined. Between-group differences (those with current impulse control disorders compared to those without) were tested using the Pearson chi-square and 2-sided Fisher exact test for categorical variables and 2-tailed independent samples t-tests for continuous variables.
Results: 41 (40.2%) of 102 patients were diagnosed with at least one current co-occurring impulse control disorder. Sixteen (15.7%) had two or more current impulse control disorders. The most common impulse control disorders were intermittent explosive disorder (n=13;12.7%), pathologic skin picking (n=12;11.8%), and kleptomania (n=9; 8.8%). Patients with co-occurring impulse control disorders were significantly more likely to have a co-occurring anxiety disorder and to have been psychiatrically hospitalized. Those with an impulse control disorder were, on a trend level, more likely to be female. Female adolescents were significantly more likely to have pyromania. No other gender differences were noted.
Discussion: Impulse control disorders appear common among adolescent psychiatric inpatients. Additional larger studies are needed to examine the prevalence of impulse control disorders among adolescents in the general population and specific psychiatric groups.

146. Classical Conditioning Using Transcranial Magnetic Stimulation as the Unconditioned Stimulus

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Sponsor: James Ballenger

Background: TMS depolarizes neurons in brain cortex via a magnetic field generated externally over the scalp. This technique is safe for human research when used within safety guidelines. With classical conditioning, an unconditioned stimulus naturally elicits an unconditioned response. The unconditioned stimulus is then paired with a conditioned stimulus, such that the conditioned stimulus alone generates a conditioned response (generally resembles the unconditioned response). We are beginning to explore whether the TMS technique fits into the classical conditioning framework. Specifically, we have examined whether TMS over motor cortex can serve as the unconditioned stimulus, with the motor evoked potential as the unconditioned response.
Methods: TMS is repeatedly paired with a compound visual/auditory stimulus. Various parameters such as number of pairings and time between pairings are explored. The compound conditioned stimulus is then presented alone, with the EMG recordings assessed for possible conditioned responses. We also examine the response to presentation of the unconditioned stimulus alone, after the pairing contingency has been established.
Results: Maximizing the number of pairings, with a reduced time between pairings, appears to result in conditioning. Approximately 40% of participants with these parameters show at least one conditioned response (albeit much weaker than the unconditioned response) to several conditioned stimulus tests. Presentation of the unconditioned stimulus alone, following establishment of the pairing contingency, generates a consistently robust motor evoked potential (on the order of 2-fold stronger than the unconditioned response prior to pairing).
Discussion: Early results indicate that TMS may have properties that fit within the classical conditioning framework. As classical conditioning characteristically involves the pairing of two sensory stimuli, TMS provides a method for exploring brain behavior in other systems. Such work may lead to more efficient and effective treatments, as well as establish a screening mechanism for drugs that affect associative learning.

147. A Prospective, Open-Label Study of Aripiprazole in Youth with Asperger's Disorder and Pervasive Developmental Disorder Not Otherwise Specified

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Asperger's disorder and pervasive developmental disorder not otherwise specified (PDD NOS) are lifelong PDDs characterized by severe impairments in social interaction and communication, in addition to restricted patterns of behavior, interests, and activities. Moreover, maladaptive behaviors such as aggression, self-injurious behavior, and irritability are commonly observed and can have a devastating impact on the affected individual and his or her family. This prospective, open-label study sought to determine the effectiveness, tolerability, and safety of aripiprazole for the treatment of interfering behaviors in children and adolescents with Asperger's disorder and PDD NOS.
Methods: This is a prospective, 14-week open-label investigation of aripiprazole in 25 children and adolescents with Asperger's disorder or PDD NOS. Subjects were required to be free of all psychotropic medications for at least two weeks (four weeks for fluoxetine), and have a Clinical Global Impression– Severity (CGI-S) score of at least 4 and a score ≥ 18 on the Irritability Subscale of the Aberrant Behavior Checklist (ABC). All subjects initially received 1.25 mg/day of aripiprazole. The investigators increased the dosage to a maximum of 15 mg/day over 4 weeks, if optimal clinical response had not occurred and intolerable adverse effects had not emerged. The dosage maintenance phase lasted 8 weeks. Primary outcome measures included the CGI-S, CGI-Improvement (I), and the Irritability subscale of the ABC.
Results: To date, 13 subjects, 3 females and 10 males, aged 5-17 years (mean, 8.9 years), have completed the study. Full scale intelligence quotient (IQ) scores ranged from 53 to 112, with a mean score of 78. The final dosage of aripiprazole ranged from 2.5-15 mg/day (mean, 7.5 mg/day). Twelve (92.3%) of 13 subjects responded, based on a CGI-I score of 1 or 2 (very much or much improved) and a ≥ 25% improvement on the ABC. Baseline ABC-Irritability subscale scores ranged from 18 to 40 (mean, 29.5), whereas week 14 scores ranged from 0 to 27 (mean, 6.8). The mean CGI-I at endpoint was determined to be 1.8, with 12 of 13 subjects judged to be much or very much improved in regards to interfering symptoms of aggression, SIB, and irritability. The CGI-S score decreased from an average of 4.5 ("moderately ill") at baseline to 3.4 ("mildly ill") at week 14. Aripiprazole was well tolerated. No clinically significant extrapyramidal symptoms or changes in blood pressure or heart rate were recorded. Ten subjects experienced mild tiredness. Seven subjects gained weight and 2 subjects lost weight during the study (mean, +2.7 lbs; range, -2.1 to +7.7 lbs).
Discussion: Preliminary data suggest that aripiprazole may be an effective and well-tolerated treatment for maladaptive symptoms in pediatric patients with Asperger's disorder or PDD NOS. In light of research suggesting that a dysregulation of DA and 5-HT contributes to maladaptive behavior in PDDs, aripiprazole's unique mechanism of action as a partial DA D2 and 5-HT1A agonist, and 5-HT2A antagonist, may prove to be important for both its effectiveness and tolerability. Controlled research and longitudinal studies are needed to further determine the efficacy and tolerability of aripiprazole in this understudied population.

148. The Role of Pharmacogenetics in Youth with Maladaptive Aggression

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Sponsor: Past Travel Awardee, PMRTP, 2004

Background: Aggression in youth can be present across various psychiatric diagnoses ranging from neuropsychiatric syndromes to mood, anxiety and attentional problems. Bjork et al. found that in subjects selected for having a first-degree relative with primary unipolar depressive disorder, plasma GABA was negatively correlated with aggressiveness. These data suggest that low GABA levels may correlate with some aspects of aggressiveness and may be genetically regulated. GABA A receptors are the principal inhibitory neurotransmitter receptors in the brain. They are the site of action of many clinically important drugs. Molecular evolution has given rise to many genetic variants of GABAAR subunits, including alpha 1-6, beta 1-4, gamma 1-4, delta, and rho 1-2.Chromosomal mapping studies have revealed that several of the GABAA receptor subunit genes appear to be organized as clusters. One such cluster, which consists of the GABAA receptor beta 3 (GABRB3) and alpha 5 (GABRA5) subunit genes, is located in chromosome 15q11-q13 and a second cluster is located on 5q34-q35.Studying predictors of response to aggression in children and families is an important and much needed area that requires further research.
Methods: Participants aged 7-18 and their biological family members were interviewed using the WASH-U KSADS. There were 10 subjects (mean age 11.5; 9 males/1 female) and 9 controls (mean age 11.6; 4 males/5 females). Assessment instruments included: Overt Aggression Scale (OAS), Brown-Goodwin History of Lifetime Aggression (HLA), State-Trait Anxiety Inventory for Children (STAIC), Children's Depression rating Scale -Revised (CDRS-R), SCID (The Structural Clinical Interview), Hamilton Depression and Anxiety Scales.
Results: Descriptive Statistics, chi-square test (Fischer's Exact Test when n was less than 5 in either control or non-control group) and t-tests were used to examine the difference in quantitative (self-report measures) and qualitative data (structured interviews) between groups. Two tail t-tests were used with a significant level set at ($p = <.05$). Significant differences were found between subjects and controls on HLA total ($p=.003$), CDRS total ($p=.006$) and OAS ($p=.042$). Differences were also noted on the GAF current score ($p<.001$). Fisher's Exact Test showed significant differences on current diagnoses of ODD ($p=.011$) and Disruptive Behavior Disorder NOS ($p=.033$) among the two groups. A significant difference between parents of controls and parents of subjects was found on the HLA total score ($p=.002$). Blood and saliva samples collected for genotyping were not analyzed given the very small sample size.
Discussion: Aggression in youth can present across various psychiatric diagnoses ranging from neuropsychiatric syndromes to mood, anxiety and attentional problems. This problem causes significant dysfunction in the school and home settings. By understanding if aggression runs in families, interventions from a psychopharmacological and psychosocial standpoint can be implemented which can prove to be very useful in improving the overall functioning and quality of life for the entire family. As suggested by the results of the very small sample in this study, subjects with disruptive behaviors present with a longer history of lifetime aggressive behaviors, more depressive symptoms and have an overall lower level of global functioning. The significant difference found between parents of controls and parents of subjects in the HLA total score ($p=.002$), is suggestive of aggressive behaviors running in families. This is a very limited sample, hence genotyping of blood and saliva samples collected was not conducted as the results will not be conclusive. Studies conducted with a much larger sample are required to provide conclusive evidence regarding the predictors of response to aggression in children and families.

149. Catecholamines and a Beta-Adrenergic-Like Receptor in the Cellular Slime Mold, *Dictyostelium discoideum*, Along with Possible Implications for the Evolution of Multi-Cellular Life Forms

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Background: *Dictyostelium discoideum*, a eukaryotic cellular slime mold, undergoes a developmental regimen wherein individual vege-

tative cells aggregate upon starvation to form multi-cellular fruiting bodies that generate spores. *Dictyostelium* is known to possess as many as fifty-five G protein-coupled receptors (GPCRs), including twelve binding cAMP, and one each for GABA and glutamate, the latter two reported recently. Here we demonstrate the existence of another GPCR, one that is apparently a generic catecholamine receptor.
Methods: Radioisotope (tritium) receptor-ligand affinity studies of *Dictyostelium* and high pressure liquid chromatography (HPLC) for catecholamines and indolamines.
Results: Receptor-ligand affinity studies of *Dictyostelium* established specific binding of norepinephrine (NE; Kd=6 x 10-6 M), epinephrine (Epi; Kd= 1 x 10-6 M), and dopamine (NE; Kd = 6 x 10-6 M). Further, this receptor showed specific drug affinities against [H-3]-NE that are suggestive of a beta-adrenergic profile: 1) isoproterenol (beta-agonist, Kd = 3 x 10-6 M), 2) propranolol (beta-antogonist, Kd = 3 x 10-6 M); in contrast 3) prazosin (alpha-1-antagonist, Kd = 1 x 10–3 M), 4) clonidine (alpha-2-agonist, Kd > 10-3 M). Via HPLC we demonstrated that *Dictyostelium* possesses high levels of NE and Epi throughout its 24-hour life cycle, with markedly more during the single cell, vegetative state than during the spore-forming, multi-cellular stalk stage. 5-HT was also detected, but not DA, octopamine or melatonin via our HPLC methods here.
Discussion: We are presently determining the effects of beta-type NE agonists and antagonists on cAMP levels and glucose metabolism in both these *Dictyostelium discoideum* developmental stages, as well as examining potential roles of catecholamines in *Dictyostelium's* transition from one developmental phase to another. We are also attempting to clone this catecholamine receptor. That DA is present at low levels at best, but NE and EPI are high, the receptor probably functions as an adrenergic, beta-like receptor, despite DA's affinity. That octopamine was not detectable challenges some theories that it is "ancestral" to NE and Epi. More generally, that a single-celled organism has apparently developed catecholamine functions may have profound implications for theories of catecholamine evolution. The role of catecholamines, if any, in *Dictyostelium's* transitions from single to multi-cellular life stages remains to be elucidated, but is likewise of critical evolutionary interest.

150. Omega-3 Fatty Acids in Psychiatry: An American Psychiatric Association Subcommittee Report on the Evidence and Recommendations for Future Research

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Sponsor: Katherine L. Wisner

Background: The authors of this paper were invited to participate by the Committee on Research on Psychiatric Treatments of the American Psychiatric Association. Our charge was the evaluation of the evidence base for the therapeutic use of omega-3 essential fatty acids (n-3 EFA) in the treatment of psychiatric disorders. We report on: 1) considerations of the biological plausibility of the role of n-3 EFA in psychiatric disorders; 2) a diagnosis-specific critical evaluation of information related to n-3 EFA biochemical status, prevention, and treatment; and 3) recommendations for future research. We reviewed the literature on n-3 EFA in psychiatric disorders to provide clinically relevant evidence-based information to psychiatrists. We sought to determine if the available data support the use of n-3 EFA for use in the prevention and/or treatment of psychiatric disorders.
Methods: Specific disorders reviewed included major depressive disorder, bipolar disorder, schizophrenia, dementia, borderline personality disorder and impulsivity, and attention deficit with hyperactivity disorder. Meta-analyses were conducted in major depressive and bipolar disorders and schizophrenia, as sufficient data were available to conduct such analyses in these areas of interest.
Results: The preponderance of epidemiological and tissue compositional studies supports a protective effect of n-3 EFA intake, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in affective disorders. Meta-analyses of randomized controlled trials demonstrate a statistically significant benefit in unipolar and bipolar depression (p=0.02). The results were highly heterogeneous, indicating that it is important to examine the characteristics of each individual study to note the differences in design and execution. There is less evidence of benefit in schizophrenia.
Discussion: EPA and DHA appear to have some potential benefit and negligible risks in major depression and bipolar disorder, but results remain inconclusive in most areas of interest in psychiatry. Treatment recommendations and directions for future research are described. Health benefits of n-3 EFA may be especially important in patients with psychiatric disorders, due to high rates of smoking and obesity, and the metabolic side effects of some frequently utilized medications.

151. The Effect of Cold-Immobilization Stress on Human Saliva N-Acetylserotonin

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Background: Cold-immobilization stress stimulated rat pineal synthesis of N-acetylserotonin, the immediate precursor of melatonin. The effect of cold-immobilization stress on human pineal indoles metabolism is not known.
Methods: N-acetylserotonin levels (ELISA) were studied in 17 human volunteers subjected to 2 hours of cold exposure in resting positions. Saliva samples were collected before and the end of the procedure.
Results: N-acetylserotonin saliva levels were on average 20 %% higher at the end of cold-immobilization than at the baseline ($p< 0.01$).
Discussion: The obtained results indicate that cold-immobilization stress stimulates the N-acetylserotonin production in humans similar to earlier established effect in laboratory animals.Both N-acetylserotonin and melatonin play a major role in immune response participating in T- cell action.Considering the involvement of immune systems in the development of various psychiatric conditions (such as schizophrenia, depression, vascular cognitive impairment)the present data might contribute to the understanding of pathophysiological mechanisms of psychiatric disorders.

152. Does Concomitant Mood Stabilizer Therapy Affect Long Term Metabolic Profile in Schizophrenia and Bipolar Disorder? Results of a Long-Term Naturalistic Comparison of Olanzapine and Risperidone

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Sponsor: Frederick Goodwin

Background: Most studies of the effect of olanzapine (OLZ) and risperidone (RIS) on metabolic measures have been cross sectional in nature, with limited consideration of diagnosis, dosage, or concomitant medications on BMI, glucose (GLU), or lipid measures. The time course of metabolic changes has important implications for frequency of monitoring. This study attempted to fill some of these needs.
Methods: 187 patients who had not recently been treated with OLZ or RIS, with diagnoses of schizophrenia (SCH) or schizoaffective disorder (SAD; n = 87) or bipolar disorder (BPD) (n = 100) were randomized to OLZ (to 20 mg/day) or RIS (to 6 mg/day). 24 patients received valproate (VAL) on clinical grounds, 13 in the RIS group, 11 in the OLZ group. Another 29 patients received various other mood stabilizers (MS). Metabolic measures (weight, BMI), fasting blood GLU

(FBG), glycosylated hemoglobin (HgbA1c), total cholesterol, low and high density lipoprotein (LDL, HDL), triglycerides (TG), and the ratio of TG to HDL (TG/HDL) were obtained in a fasting state at baseline, 1, 3, 6 and 12 months. Data were analyzed with a mixed model analysis of covariance with mood stabilizer (MS) treatment as a between subjects, fixed effect factor. The six month data and the TG/HDL ratio, a useful measure of syndrome X and cardiac risk (upper limit of normal, 3.5), will be emphasized.
Results: For all subjects, BMI, TG, and the TG/HDL ratio increased, beginning at one month, in the OLZ-treated patients, with the peak increases occurring at six months. Only weight and BMI increased with RIS but those increases were significantly smaller than those for OLZ. At 6 months, RIS patients treated with no MS was associated with a TG/HDL ratio of 4.3± (SEM) 0.5 compared with 3.0±0.9 with VAL and 5.1±0.9 with other MS. OLZ monotherapy was associated with a 6 month TG/HDL ratio of 5.4±0.4 compared with a ratio of 5.9±0.9 with VAL and 9.4±0.8 with other MS. OLZ in combination with other MS was significantly greater than the other two OLZ groups. None of the RIS groups were significantly different from each other. There were no significant differences between BPD patients and those with SCH or SAD, in any of the measures, with the exception of higher HDL levels in the BPD patients ($p = 0.04$). Increases in lipids in the OLZ-treated patients were independent of the increases in BMI. Three new cases of diabetes mellitus (DM) developed with RIS and four with OLZ. The increments in BMI and lipids were independent of the doses of OLZ or RIS.
Discussion: The results reported here confirm that OLZ is significantly more likely than RIS to adversely affect metabolic status and that this is as likely to occur in patients with BPD as in those with schizophrenia. The greater adverse effect of OLZ on metabolic measures compared to RIS is more evident in patients who receive concomitant treatment with some MS other than VAL. There were too few subjects with other MS to draw firm conclusions as to which appeared to be augmenting the effects of OLZ but inspection suggested carbamazepine and topiramate had the greatest influence. The peak increases in BMI, lipids, and GLU measures were found at 6 months, suggesting an evaluation of metabolic status at this time is indicated for follow-up of metabolic measures in clinical practice. The TG/HDL ratio provided the best single measure of adverse metabolic adverse effects. More intensive study of the interaction between specific MS and atypical Pads appears warranted because of the suggestive results reported here and the frequent co-administration of these classes of drugs.

153. Dorsal Caudate Predicts Antipsychotic Response

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Background: FDG-PET studies have generally demonstrated that unmedicated patients with schizophrenia had lower relative metabolic rates in the striatum than normal controls and that antipsychotic medication raised metabolic rates.
Methods: We have carried out three FDG-PET studies which indicate that low relative metabolic rates in the dorsal caudate nucleus at baseline are indicative of greater clinical response to antipsychotics.
Results: Study 1: Haloperidol/placebo crossover in adult patients with schizophrenia. Twenty-five patients with schizophrenia entered a double-blind crossover trial of haloperidol and placebo. Patients received either placebo or medication for the first 5 weeks and they received the other treatment for the second 5 weeks. FDG-PET scans were obtained at weeks 5 and 10. Patients with low relative metabolic rates in the caudate nucleus while they were receiving placebo were more likely to show decreases in their Brief Psychiatric Rating Scale scores with haloperidol treatment than individuals with normal or high metabolic rates. Study 2: Risperidone in obsessive-compulsive disorder. We studied 15 nondepressed patients with obsessive-compulsive disorder (OCD) who were nonresponders to serotonin reuptake inhibitors with an additive trial of risperidone. Patients with low relative metabolic rates in the striatum were more likely to show a clinical response. Study 3: Olanzapine and haloperidol in never-medicated adolescents. We acquired FDG-PET in 30 never-previously medicated psychotic adolescents (ages 13-20). Low metabolic rates in the caudate predicted haloperidol response on the BPRS total score (dorsal right caudate at baseline vs. BPRS total change $r=-0.64$, positive symptoms $r=-0.76$) while high metabolic rates at baseline predicted olanzapine response ($r=0.41$, $p<0.05$).
Discussion: All three studies confirm the dorsal caudate relative metabolic rate as the best predictor of antipsychotic response. This dorsoventral difference is consistent with the reported greater D2 receptor density in dorsal caudate. Together these studies suggest that the predictive effect of low baseline metabolic rates in the dorsal caudate nucleus is replicable and applies in varied clinical populations.

154. Rate of Decline in Anisotropy with Age in Patients with Schizophrenia in Cross-Sectional and Longitudinal Samples

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Background: Diffusion tensor anisotropy has been found to be reduced in the white matter of the frontal lobes in patients with schizophrenia and to decrease with age in normal subjects. In this study we examine the rate of decrease with age in two samples of patients.
Methods: To examine age effects across the lifespan, we imaged a cross sectional sample of 63 patients obtained in 2003-2005. To assess longitudinal change, we imaged a sample of 23 patients imaged twice five years apart in the 1998-2006 period.
Results: Cross-sectional sample: There were a total of 119 subjects including scanned, 55 normal volunteers (32 men, 23 women, mean age 42.4) and 63 patients with schizophrenia (44 men and 19 women, mean age 41.7; age and sex group differences, ns). Anatomical images were acquired (3.0 T Siemens Allegra) using a magnetization prepared rapid gradient echo (MP-RAGE), and diffusion tensor images (DTI) were acquired using a pulsed gradient spin-echo sequence, (28 3-mm images). Images were processed using our own Matlab routines to produce fractional anisotropy (FA) images. We computed t-test images using our own R language program for the age by diagnosis interaction, testing age-regression slope differences between the groups. Regions in the orbital and dorsolateral frontal lobe and temporal lobe white matter showed significantly greater rates of anisotropy decrease in patients than controls. Longitudinal sample: A subsample of a longitudinal cohort (23 patients and 8 normal controls scanned 5 years apart) was studied with DTI. The DT acquired 14 7.5-mm-thick slices (1.5 T, EPI sequence). After alignment of both DT images to the year-1 SPGR anatomical image, significantly greater decreases in anisotropy were found in orbitofrontal cortex, anterior cingulate, and left temporal Brodmann area 20.
Discussion: Taken together, these studies suggest a more rapid anisotropy decrease in patients with schizophrenia than in controls.

155. Asenapine Displays Distinctive Induction Patterns of *c-fos* mRNA Expression in Rat Forebrain Regions

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Sponsor: Frank I. Tarazi

Background: Asenapine, a novel psychopharmacologic agent under development for treatment of schizophrenia and bipolar disorder, displays a unique human receptor signature. The aim of the present study was to map the neuroanatomical sites of response to varying doses of asenapine in the male rat forebrain, using changes in *c-fos* mRNA expression as a neuronal marker for cell activation.
Methods: After daily handling for 5 days to ensure low basal *c-fos* mRNA expression, 24 adult male rats received asenapine (0.05, 0.1 or 0.5 mg/kg subcutaneously) or vehicle (0.9% saline, 1 mL/kg). After 45 minutes, the brains were removed and processed for *c-fos* in situ hy-

bridization histochemistry. Autoradiographic results were quantified in 42 brain regions and subregions. Data were analyzed statistically by analysis of variance and Dunnett's tests. Results at each site are expressed as percentage increase in cell activation with asenapine versus vehicle.
Results: Asenapine-induced increases in *c-fos* signal varied with dose and brain region; no decreases were observed in any region at any tested dose. At 0.05 mg/kg, asenapine significantly increased *c-fos* response in the dorsolateral, dorsomedial, ventrolateral, and ventromedial quadrants of the striatum (75%–131% anteriorly; up to 88% more posteriorly) and preferentially in the shell of the nucleus accumbens (82%). At 0.1 mg/kg, activation reached 241% in the striatum anteriorly and 146% in the nucleus accumbens (shell), and 13 additional areas were activated: the medial prefrontal, retrosplenial, and parietal cortices (35%, 21%, 21%, respectively); lateral septum (43%); olfactory tubercle (21%); hippocampal CA_1 and CA_2 cell fields (40%, 28%); lateral (both subregions) and medial habenula (22%–36%); and the dorsal, medial, and ventral thalamus (15%, 30%, 19%). At 0.5 mg/kg, the retrosplenial and parietal cortices, hippocampal cell fields, and habenular and thalamic regions were no longer responsive, but significant activation was noted in the core of the nucleus accumbens (32%), hypothalamic and thalamic paraventricular nuclei (93%, 38%), and medial amygdaloid nucleus (79%). Activation in the striatum and nucleus accumbens (shell) peaked at 443% and 218%, respectively.
Discussion: Asenapine produces different patterns of neuronal activation depending on dose. Compared with established conventional and atypical antipsychotics, asenapine differed in activation of the parietal cortex, medial amygdala, hippocampal CA_2 cell field, and medial habenula. This pattern of activation suggests that asenapine may influence activity in extended neuronal circuitry believed to be relevant to the pathology of schizophrenia, including cognition, stress, and emotional state.

156. Perfusion fMRI of Limbic Activation to Cigarette Cues: Male/Female Differences and Blockade of Brain's Response with Baclofen Treatment

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Sponsor: Thomas A. McLellan

Background: Smoking is the primary cause of premature death in the US. However, pharmacotherapies to aid smokers in quitting are scarce and only partially effective. Dependence on cigarettes is maintained by nicotine and the reminders associated with it, however the influence of these factors on smoking behavior may differ between the sexes. The direct effects of nicotine on the brain may influence male smoking more while cigarette reminders (cues) may be more important in maintaining female smoking.
Methods: Thus, we utilized continuous arterial spin-labeled (CASL) perfusion fMRI to reveal the brain substrates of cigarette cue-induced craving, examine the data for male/female differences, and test baclofen, a purported anti-craving medication for its ability to blunt cue-induced activation. Twenty-one smokers (12 females) completed smoking and nonsmoking cue scanning sessions counterbalanced and preceded by smoking. Subjective craving reports were collected before and after each session. SPM2 software was employed to analyze data across conditions without masking.
Results: Perfusion was greater during smoking cues in ventral striatum, amygdalae, orbitofrontal cortex, hippocampi, thalamus, and insula ($p < 0.05$ FWE corrected). Increased perfusion correlated with intensity of cigarette craving, selectively in dorsolateral prefrontal cortex ($r2 = 0.54$) and posterior cingulate ($r2 = 0.53$). Male and female brain activity to smoking cues differed significantly ($p < 0.05$ FWE corrected). Female activation was more widespread and correlated with craving in ventral striatum, pons (pedunculopontine nucleus), thalamus, dorsolateral prefrontal cortex, anterior and posterior cingulate, and orbitofrontal cortex ($r2$ for all regions > 0.45). Male perfusion in response to smoking cues correlated with craving bilaterally in orbitofrontal cortex ($r2 = > 0.58$). Overall craving scores were significantly higher in females compared to males ($p < 0.02$). Of 21, 14 subjects were treatment seekers and were imaged after 3 weeks of baclofen (80 mg/day; N=7) or placebo (N=7) in a double blind study for 3 weeks and were imaged again. The original brain response to cues was replicated in placebo subjects. Baclofen suppressed the initial brain response to smoking stimuli in reward-related circuits such as bilateral amygdalae, ventral striatum, insula and dorsolateral prefrontal cortex ($p < 0.007$,corrected), with t values ranging from 7.5 to 17.8.
Discussion: This study corroborates over thirty years of animal research on the neural correlates of conditioned drug reward and is the first to correlate the intensity of craving with neural activation in ventral striatum, a critical reward substrate, and with the interconnected amygdalae, cingulate, and orbitofrontal cortex. Further, this report supports the hypothesis that females respond differently to cues, a finding that may have critical implications for future therapeutic strategies. The baclofen finding emphasizes the importance of continuing to study its features to determine if its ability to reduce smoking behaviors corresponds with its ability to quiet the brain. If so, the CASL perfusion fMRI cue-induced craving paradigm here could be used to quickly screen other potential medications for their efficacy as anti-craving/relapse smoking cessation aids.

157. Risk for Diabetes and Primary CHD in Schizophrenic Patients Treated With Atypical Antipsychotics: An Analysis of CATIE Data Using NNH Methodology

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Background: A published study using baseline fasting laboratory values from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study indicated increases in coronary heart disease (CHD) risk among patients receiving treatment with antipsychotic agents compared with age- and sex-matched controls.[1] We used a simulation model that was based on the Framingham risk equations and national epidemiologic studies of diabetes risk to determine whether metabolic changes observed in CATIE following treatment with atypical antipsychotic agents translated into further long-term increases in CHD and diabetes risk.
Methods: Risk equations were used to estimate 10-year rates of diabetes and CHD using baseline and follow-up data from phase 1 of CATIE. Exposure-adjusted mean change from baseline was used to estimate the relative risk (RR) and number needed to harm (NNH) for olanzapine, quetiapine, and risperidone relative to ziprasidone, and for ziprasidone relative to no treatment.
Results: Olanzapine, quetiapine, and risperidone were associated with an increased risk of diabetes and primary CHD when compared with ziprasidone. In descending order of severity the RR and NNH values were as follows: olanzapine, RR = 1.17 and NNH = 31 for diabetes and RR = 1.09 and NNH = 120 for primary CHD; quetiapine, RR = 1.06 and NNH = 88 for diabetes and RR = 1.07 and NNH = 152 for primary CHD; risperidone, RR = 1.06 and NNH = 92 for diabetes and RR = 1.04 and NNH = 294 for primary CHD. Treatment with ziprasidone was not associated with an increased risk for diabetes or CHD (RR < 1.00) compared with no treatment.
Discussion: These results are consistent with the ADA/APA consensus statement regarding the long-term safety of atypical antipsychotic agents and provide further evidence that ziprasidone treatment does not increase risk for diabetes and CHD above the baseline levels observed in CATIE. **Reference** 1.Goff DC, Sullivan LM, McEvoy JP, et al. A comparison of ten-year cardiac risk estimates in schizophrenia patients from the CATIE study and matched controls. *Schizophr Res.* 2005;80:45–53