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Poster Session I

1. Neuropsychological Performance and Vascular Function in Patients with Atherosclerotic Vascular Disease

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Forearm vascular function is reflective of general vascular health in patients with atherosclerotic vascular disease (AVD). Indeed, all known risk factors for AVD are also associated with reduced vascular function. We previously reported preliminary data (N = 14) that demonstrated a significant relationship between forearm vascular function and neuropsychological performance in individuals with AVD. The current study was conducted to confirm and extend those findings in a much larger, non-overlapping sample.
Methods: Participants were 82 individuals with AVD, with no history of stroke, cardiac surgery, or dementia. Forearm vascular function was measured before and after brachial artery infusion of vasoactive agents (acetylcholine, nitroprusside, verapamil). Neuropsychological functioning was assessed with the Repeatable Battery for the Assessment of Neuropsychological Status. Statistical analysis included multiple regression and partial correlations, controlling for education.
Results: Vascular function was significantly associated with global neuropsychological performance [R Square Change = .116, F Change (3,74) = 3.72, p = .015]. Follow-up analyses indicated that smooth muscle function was the aspect of vascular function most strongly associated with neuropsychological performance. Delayed memory and semantic fluency were the aspects of neuropsychological performance most strongly associated with vascular function. Individual vascular risk factors (e.g. glucose level, blood pressure, etc.) were not significantly associated with neuropsychological performance when controlling for vascular function.
Discussion: Forearm vascular function is significantly associated with neuropsychological performance in individuals with AVD. With additional research, measures of vascular function might be useful in the early identification of individuals who are at greatest risk for developing vascular cognitive impairment.

2. A Review of the Safety and Tolerability of Treatments for Moderate to Severe Alzheimer's Disease

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Background: Safety concerns play a prominent role in selecting an appropriate Alzheimer's disease (AD) treatment. Memantine, an antagonist of N-methyl-D-aspartate receptors, is approved for treatment of moderate to severe AD in the US and Europe. Donepezil, a cholinesterase inhibitor, is approved for treatment of mild to moderate AD and is under review for approval in severe AD. This analysis compares the safety and tolerability of memantine and donepezil in the treatment of patients with moderate to severe AD.
Methods: Relevant databases and conference procedings were searched for randomized, placebo-controlled clinical trials of memantine and donepezil involving patients with moderate to severe AD, with a minimum duration of 24 weeks. Trends from each study and pooled analysis were examined.
Results: Three memantine and four donepezil trials fulfilled the search criteria. Memantine treatment was associated with a lower rate of discontinuations (overall: 20.3% vs. 27.9%) and a lower rate of discontinuations due to adverse events (AEs) (9.9% vs. 14.0%) compared to placebo, with a similar incidence of AEs (78.1% vs. 76.0%). No trend in type of AEs was observed across trials. Compared to placebo, donepezil treatment was associated with a greater rate of discontinuation in most individual trials (overall: 23.0% vs. 19.8%), a higher rate of discontinuations due to AEs (14.0% vs. 8.8%), and higher incidence of AEs (80.8% vs. 73.5%). Diarrhea was associated with donepezil treatment across trials, as was vomiting in two of the four studies.
Discussion: In treatment of patients with moderate to severe AD, donepezil use was associated with gastrointestinal symptoms. No such trends were seen in the treatment of patients with memantine. Given the low number of trials and limitations of comparing treatments between separate trials, prospective studies directly comparing memantine and donepezil would be required to properly address tolerability differences between the two drugs.

3. White Matter Integrity in Aging – Linking Vascular Risk and Cognition to the Brain

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Background: While the relationship of vascular risk factors (VRF) and vascular diseases to cognitive impairment, cognitive decline, and dementia is well established, the mechanisms linking vascular risk factors, vascular brain injury (VBI) and cognitive impairment are not well understood. We investigated the relationship between vascular risk factors, vascular brain injury and cognitive impairment using a subsample of the community based study, the Swedish National study of Aging and Care in Kungsholmen (SNACK).
Methods: The SNACK is a multidisciplinary, longitudinal study of aging targeting the age 60+ population (n = 3363). Baseline imaging data were available on a subset of 552 subjects ranging from 60 to 93 years-old. The following preliminary findings are derived from an initial analysis of a random sampling of one quarter of the 552. VRFs included life habits (diet/body mass index, alcohol, smoking), hypertension, ischemic diseases (both heart and cerebral), and diabetes and obesity as part of the metabolic syndrome. VBI, as measured by Diffusion Tensor Imaging(DTI), includes measures of mean diffusivity (MD) and fractional anisotropy (FA) of white matter in the following brain regions: centrum semiovale, frontal, and occipital. Hippocampal volume measures were also obtained as measures of neurodegeneration. Five major domains of cognitive functioning were evaluated: episodic memory, executive functioning, processing speed, verbal knowledge, and visuospatial skill.
Results: Significant relationships were observed between VRF and cognitive functioning, VRF and DTI brain measures and DTI and cognitive functioning. When DTI indices were entered into a prediction model along with demographic factors (e.g., age, gender, and education) and the composite of vascular burden, in each case the individual contribution of the vascular burden score to cognitive performance was reduced (by .032 for frontal FA and .052 for centrum semiovale MD) and the total variance accounted for by the prediction model was improved (by .036 for frontal FA and .028 for centrum semiovale MD). Hippocampal volume measurements were significant predictors of episodic memory performance, independent of age, gender, and education. No other significant relationships were found between hippocampal volume and measures of cognitive performance (including speed, visuospatial, and executive functioning). In contrast, indices of MD and FA correlated with visuospatial function but not memory.
Discussion: These preliminary results provides support for our model of white matter integrity (brain) mediating the relationship between VRF and cognition. Given the well established role of medial-temporal regions as early markers of neurodegeneration, these findings lend partial support to a hypothesized differentiation between microvascular, subcortical pathology and neurodegenerative pathology.

4. Systematic Review and Meta-Analysis of Cholinesterase Inhibitors and Memantine for Moderate to Severe Alzheimer's Disease

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Background: Context: Cholinesterase inhibitors are FDA-approved for mild to moderate Alzheimer's disease and not for severe AD (donepezil is currently under FDA review for this indication). Memantine, a non-competitive, NMDA-antagonist, is FDA- approved for the treatment of moderate to severe AD and but not for mild. Cholinergic neurons in the nucleus basalis are decreased in AD patients along with choline acetyltransferase and acetylcholinesterase, and substantially more so in severe as compared to mild AD. Objective: To assess the consistency of the efficacy and safety evidence for cholinesterase inhibitors and memantine in moderate to severe AD; and to generate hypotheses about relative efficacy and safety of cholinesterase inhibitors and memantine
Methods: Data Sources and Trials Selection: The Cochrane Controlled Trials Register (2006, Issue 3) and Specialized Dementia Register, meetings presentations, and other material were searched to identify placebo-controlled, double-blind, parallel-group, randomized clinical trials of donepezil, rivastigmine, galantamine, and memantine for patients with moderate to severe AD. Data Extraction: Trials design, clinical characteristics, cognitive, clinician global, activities of daily living (ADL), behavior scale outcomes, and adverse events were extracted.
Results: Data Synthesis: 5 donepezil, 1 rivastigmine, and 5 memantine trials were included, totaling 1391 patients in donepezil, 218 in rivastigmine, and 1436 in memantine trials. Behavior and ADL ratings reported as not statistically significant from 2 partially presented donepezil trials were withheld by the drug company, Eisai/Pfizer. Results from two memantine trials were withheld by Lundbeck and Daiichi Sankyo. Average baseline MMSE scores per trial ranged from 6.1 to 14.4 in mostly 6-month long trials. Outcomes were calculated for each drug-placebo contrast, as meta-analytic summaries for each drug and overall, and were expressed as weighted mean differences or odds ratios with their 95% CIs and P values using Cochrane Review Manager 4.2. There was overall consistent but modest statistical efficacy for donepezil and memantine on cognitive, global, and ADL outcomes. Cognitive effect sizes with donepezil were larger than with memantine. Discontinuations due to adverse events were more likely with donepezil and less likely with memantine. Overall effects on behavior rating scores were inconsistent with most trials not showing statistically significant effects. Sensitivity analyses suggested greater statistical effect on cognition and non-significant effects on behavior ratings with more severe dementia with donepezil.
Discussion: The statistically greater cognitive effect size for donepezil was offset by greater likelihood of discontinuation and adverse events compared to memantine. Cognitive effects of donepezil were statistically greater (though still modest) with increasing severity of AD while not improving overall behavior. Cognitive effects also might be greater in severe AD than in milder AD and consistent with the greater extent of cholinergic deficits. Direct comparisons are needed of memantine and donepezil alone and in combination. Individual patient data should be made available, and companies should fully disclose their clinical trials results to better inform patients on efficacy and safety.

5. Bupropion for the Treatment of Methamphetamine Dependence

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Background: Bupropion, an approved antidepressant with monoamine uptake inhibition properties and mild stimulant effect, was tested in a double-blind placebo-controlled study for the treatment of methamphetamine dependence.
Methods: 151 participants with DSM-IV diagnosis of methamphetamine dependence were consented and enrolled. 72 participants were assigned to placebo and 79 to Bupropion SR 150mg BID. Patients were required to come to the clinic three times per week for assessments, urine drug screens, and group psychotherapy. The primary outcome was the change in proportion of participants having a methamphetamine-free week. Secondary outcomes included Addiction Severity Index (ASI), craving, and quantitative urine methamphetamine.
Results: Analysis of the primary outcome showed a trend toward significance for the total sample ($p=0.09$), favoring bupropion. Baseline use in the 30 days prior to screening, using time-line follow back, differentiated the total sample into low/moderate users (<18 days, n=71) and high users (>18 days, n=80). Bupropion showed a statistically significant effect in the low/moderate use subgroup compared to placebo ($p=0.03$), with a greater increase in the number of non-use weeks over the treatment period. This subgroup also had significantly reduced mean quantitative methamphetamine in urine. High users did not have similar effects. Gender analysis for the total sample showed that males (n=101) had a significant effect favoring bupropion treatment ($p=0.03$), while females (n=50) did not. Co-morbid conditions, including depression and attention deficit disorder, did not change the findings.
Discussion: These data suggest that bupropion, in combination with behavioral group therapy, was effective for the treatment of participants with low/moderate methamphetamine dependence.

6. An Evaluation of Gender Differences in Response to Cues in Cocaine-Dependent Individuals

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Sponsor: Kathleen Brady

Background: A critical area in the investigation of gender differences in cocaine dependence is differences in factors influencing initiation, maintenance and relapse to drug use. A number of studies investigating attributions of drug use and relapse in substance-dependent individuals have reported that women are more likely to report substance use associated with negative emotional states and interpersonal distress, whereas men are more likely to report use associated with external stimuli.
Methods: In this investigation, subjective and HPA axis (e.g., ACTH, cortisol) response to the presentation of cocaine-related cues and a psychological stress task (Trier Social Speaking Task; TSST) are being compared in cocaine-dependent men and women and matched control groups without cocaine dependence. A CRH stimulation test is also being performed. It was hypothesized that cocaine-dependent women would have a more robust craving response to a social stressor and cocaine dependent men would have a more robust craving response following cocaine related cues.
Results: To date, 150 subjects have been recruited, and 76 have completed the protocol. Both cocaine groups experienced higher stress ($p=0.04$) and craving ($p<0.0002$) after cocaine cue exposure as compared to the control groups. There was no gender difference in craving or stress response to cocaine cues in cocaine dependent individuals. While there was a trend towards greater peak change ACTH response following cue in cocaine-dependent men as compared to women ($p=0.07$), no gender difference was observed for cortisol following cue presentation. All groups had increased stress ratings after the TSST, but only the cocaine groups reported craving following the TSST ($p<.0002$). The Mann-Whitney-Wilcoxon test was performed to examine gender differences on the peak change in subjective responses to TSST. There is marginal evidence that cocaine-dependent women experienced more stress ($p=0.06$) and more craving ($p=0.08$)

following the TSST than cocaine-dependent men. The nonparametric sign test was used to compare the cocaine cue exposure and TSST within cocaine-dependent males and females separately. For cocaine-dependent women there were no significant differences between response to cocaine cues or TSST for either craving or subjective distress. However, for cocaine dependent men, there was significantly higher craving after exposure to cocaine cues as compared to the TSST (median difference 1.5, p=0.02). No gender differences in peak change ACTH or cortisol were found following TSST. Both cocaine groups experienced higher craving after CRH infusion as compared to the control groups (p=0.0002). There were no gender or group differences for stress or ACTH/cortisol response to CRH.
Discussion: Data collected to date suggests a greater response to a psychological stressor for cocaine-dependent women as compared to cocaine-dependent men. As predicted, for cocaine dependent men, there was significantly higher craving after exposure to cocaine cues as compared to the TSST. In addition, cocaine-dependent men and women had a greater craving response to CRH than the control group suggesting a sensitivity to a pharmacological stressor. These findings may help inform gender-specific approaches to relapse prevention in cocaine-dependent individuals.

7. Dysregulation of Signal Transduction Accompanies AMPA Receptor Trafficking in the Nucleus Accumbens During Behavioral Sensitization to Cocaine

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Sponsor: Marina E. Wolf

Background: There is substantial evidence suggesting addiction is a form of glutamate-dependant neuronal plasticity, involving cellular mechanisms similar to those mediating activity-dependent plasticity. Studies in the hippocampus have shown that regulated AMPA receptor (AMPAR) trafficking is responsible for the changes in synaptic strength seen in LTP and LTD. Using cultured neurons, we have established that dopamine (DA) receptors modulate AMPAR trafficking, suggesting a way for DA-releasing psychomotor stimulants to influence plasticity.
Methods: Drug Treatment and Behavioral Evaluation: Male Sprague Dawley rats were given 7 daily injections of either cocaine or saline (i.p.). On days 1 and 7, rats in the cocaine group received 15 mg/kg cocaine. On days 2-6, they received 30 mg/kg cocaine. Rats were killed after 7 or 14 days of withdrawal. Locomotor activity was monitored on days 1 and 7 using photobeam frames. Crosslinking and Tissue Preparation: Brains were removed rapidly and the NAc was dissected on ice from a 2mm coronal section. NAc slices (400 um) were prepared with a tissue chopper. Slices were crosslinked with BS3 and then quenched with glycine. Tissue was homogenized in lysis buffer by sonication and then centrifuged. The supernatant was then aliquotted and stored at -80°C. Western Blotting: Samples were loaded onto gradient (4%-15%) SDS-PAGE or gradient (3-8%) Tris-Acetate gels. Gels were transferred to PVDF membrane for immunoblotting. After blocking, membranes were incubated with antibodies to AMPA receptor subunits (GluR1, GluR2, GluR2/3) or kinases (pERK1/2, ERK1/2, pCaMKII, CamKII, and PKA substrates). Membranes were then washed and incubated with HRP-conjugated secondary antibodies. After washing, membranes were immersed in chemiluminescence (ECL) detecting substrate and exposed to HyperFilm ECL film. The diffuse densities of bands were determined using TotalLab (Nonlinear Dynamics Ltd., Newcastle, UK).
Results: In the present study, we found an increase in GluR1 S/I ratios and MAPK activity in cocaine sensitized rats after 7 days of withdrawal. There was no change in CaMKII activity at this time point. Correlational analysis revealed a positive relationship between GluR1 S/I ratios and MAPK activity as well as GluR1 S/I ratios and CaMKII activity in saline treated rats. These relationships were not present in cocaine treated rats.
Discussion: We recently reported an increase in AMPAR surface expression in the NAc 21 days after discontinuing cocaine that correlated with the magnitude of behavioral sensitization (Boudreau & Wolf, J Neurosci 25:9144-51, 2005). The increase in AMPAR surface expression was accompanied by an increase in the activation of signal transduction pathways known to be important for regulation of AMPAR trafficking in the hippocampus: MAPK and PKA. However, correlational analyses revealed that although saline treated rats possessed predicted relationships between these kinases and AMPAR surface expression based on the LTP literature, cocaine treated rats did not. In this study, similar results were found 7 days after discontinuing cocaine. These data suggest that signaling pathways involved in AMPAR trafficking are not merely augmented after chronic cocaine treatment, but are dysregulated. This dysregulation is present after 7 days of withdrawal from repeated cocaine and is maintained for several weeks. To further the understanding of the dynamics of this dysregulation, AMPAR surface expression, PKA, CaMKII, and MAPK activities are being evaluated in NAc cross-linked tissue obtained from 14 days after discontinuing chronic cocaine treatment.
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8. Association of Single Nucleotide Polymorphisms (SNPs) in Glutamate Receptor Genes with Theta Power of Event-Related Oscillations

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Sponsor: Henri Begleiter

Background: Endophenotypes (or intermediate phenotypes) reflect more proximal effects of genes than diagnostic categories, and hence they provide a more powerful strategy in searching for genes involved in complex psychiatric disorders. There is solid evidence for the P3 (P300) amplitude of the event-related potential (ERP) as an endophenotype for the risk of alcoholism and other disinhibitory disorders. The P3 component is not unitary, but consists of superimposed event-related oscillations (EROs) of different frequency bands, primarily frontal theta (4-5 Hz) and posterior delta (1-3 Hz). The major neurochemical substrates contributing to theta and delta rhythms and P3 involve strong GABAergic, cholinergic and glutamatergic system interactions. We already have evidence that a cholinergic muscarinic receptor gene (CHRM2) is involved in event-related theta oscillations underlying the P3. The aims of this study were to assess the potential associations between SNPs in glutamate receptor genes and the quantitative trait of event-related theta band energy during processing of target visual signals.
Methods: A subset sample of the multi-site Collaborative Study on the Genetics of Alcoholism (COGA) comprising 1,049 Caucasian subjects (from 209 families with 462 individuals diagnosed as alcohol dependent by DSM-IV) was included in genetic association analyses using the Quantitative Pedigree Disequilibrium Test (QPDT). Neural activities were recorded from scalp electrodes during a visual oddball task in which rare targets elicited P3s. The phenotype analyzed was the energy in the event-related theta band (4-5 Hz) underlying P3. Publicly available databases (NCBI) were used to identify SNPs within the candidate glutamate receptor genes, and genotyping was performed using a standard pyrosequencing method.
Results: Significant associations ($p<0.05$) were found between the event-related frontal theta power to target visual stimuli with multiple SNPs in GRM8 gene located at chromosome 7q31.3-q32.1, near the CHRM2 gene on chromosome 7q31-q35, in GRM4, near GABBR1 on chromosome 6p, and GRIK2 (GLUR6) gene, near GABRR2, on chromosome 6q.
Discussion: Our results suggest that glutamate receptor genes may be involved in modulating event-related theta oscillations in frontal re-

gions during information processing. It remains to be determined if genetic variations in the glutamate receptor genes are also involved in vulnerability to alcoholism and related disorders. These findings underscore the utility of using electrophysiology and the endophenotype approach in the molecular genetic study of psychiatric disorders.

9. Variation at the Rat Crhr1 Locus and Sensitivity to Relapse into Alcohol Seeking Induced by Environmental Stress

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Sponsor: Kjell Fuxe

Background: Gene-environment interactions are commonly implied in alcoholism and propensity to relapse, but their exact nature is presently unknown. Clinically, alcoholism is commonly co-morbid with anxiety disorders and depression, conditions characterized by maladaptive responses to stress. Behavioral analysis has long pointed to stress as one of the major environmental stimuli with an ability to trigger relapse in susceptible individuals. It is unclear whether, in alcohol dependent individuals, these stimuli trigger relapse by interacting with pre-existing genetic susceptibility factors, acquired central nervous system (CNS) neuroadaptations secondary to a prolonged history of alcohol use, or both. Here, we used an established selection based animal model, the Marchigian-Sardinian Preferring (msP) rat, which has been bred for high alcohol preference for many generations originating from Wistar rats. These lines may offer a particularly useful model of genetic susceptibility to stress-mediated relapse, since anxiety and depression-like traits have co-segregated with high alcohol preference during the selection leading to their creation.
Methods: Male Wistar and msP rats were used. Behavioral sensitivity to stress was tested in the conditioned emotional response paradigm, alcohol seeking behavior in the stress-induced reinstatement test. Expression of twenty stress-related genes in forebrain regions of msP and Wistar rats was compared by antisense riboprobe based in situ hybridization. Corticotropin-releasing rormone receptor 1 (CRH-R1) receptor binding was assessed by receptor autoradiography using [125I]-sauvagine binding in the presence of CRH-R1/R2 antagonist astressin and CRH-R1 agonist stressin1. Sequence analysis of the rat Crhr1 promoter was done using standard techniques and haplotypes were reconstructed using the Haploview program. Antalarmin, a selective CRH-R1 antagonist was administered intraperitoneally 30 min before behavioral testing.
Results: In line with previous observations we found increased behavioral sensitivity to stress in msP rats, which showed a markedly higher level of freezing during both acquisition ($F1,12=132.9$, $p<0.001$) and recall ($F1,12=6.10$, $p<0.05$) of fear conditioning as compared to Wistar rats. Pain thresholds, a potential confound, were determined using a hot plate test, but were identical in both lines. We found a lowered threshold for stress-induced reinstatement of alcohol seeking in msP rats (reinstatement at 0.3 to 0.6 mA footshock intensity in msPs, $p < 0.05$; at 0.6 to 1.0 mA in Wistars, $p < 0.05$). An innate up-regulation of the Crhr1 transcript was found in several limbic brain areas of msP rats genetically selected for high alcohol preference ($H1,231=12.5$, $p<0.001$), was associated with genetic polymorphism of the Crhr1 promoter ($\chi 2=29.53$, $p<0.00001$), and was accompanied by increased CRH-R1 density ($F1,126=125.5$; $p<<0.001$). A selective CRH-R1 antagonist (antalarmin, 10 - 20 mg/kg) was devoid of effects on operant alcohol self-administration in unselected Wistar rats, but significantly suppressed this behavior in the msP line ($F2,6=3.9$; $p<0.05$). Stress induced reinstatement of alcohol seeking was not significantly affected by antalarmin in Wistar rats, but was fully blocked in msP animals ($F2,19=6.5$ $p<0.001$).
Discussion: These data demonstrate that Crhr1 genotype and expression interact with environmental stress to reinstate alcohol seeking behavior and provide a functional validation for antagonism at CRH-R1 receptors as a mechanism for novel treatments aimed at relapse prevention in susceptible individuals.

10. Can the Gaba B Agonist Baclofen Prevent Limbic Activation by Both "Seen" and "Unseen" Cocaine Cues?

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Background: Recently, we have demonstrated that both visible 500 msec cocaine cues and even "unseen" 33 msec cocaine cues (presented outside awareness) are sufficient to activate reward-relevant limbic brain regions in cocaine patients. The early brain response to brief "seen" and "unseen" cues likely has functional significance: patients with a stronger response in these regions have greater positive affect to easily visible cocaine cues in an (off-magnet) priming task on the day after scanning. The "early" brain response to signals for drug reward may be a source of relapse vulnerability, but its neurochemical basis is not yet known. Preclinical research — and our own recent PET collaborative study with C-11 raclopride — points to endogenous dopamine release as a likely substrate for the brain response to drug cues. The GABA B agonist baclofen modulates dopamine release and reduces drug (cocaine, heroin, nicotine) motivation in animals. We thus hypothesized that baclofen might help prevent the limbic brain response to both "seen" and "unseen" cocaine cues.
Methods: Patients (n=16) were randomized to 60 mg baclofen (20 mg t.i.d.) or placebo in a residential pretreatment setting for a minimum of 7 days prior to scanning. We then used randomized, event-related BOLD (Blood Oxygen-Level-Dependent) fMRI (functional magnetic resonance imaging) at 3 Tesla to measure the brain response to cocaine-related, appetitive (sexual), aversive (injury or disease), or neutral cues in two tasks: 1) an "unseen" cue task using cues of 33 msec duration (each "backward-masked" by a 467 msec neutral stimulus) , and 2) a task using visible cues of 500 msec duration. 24 unique stimuli (in each of the 4 categories, presented twice), plus 48 null events, were "jittered" [average inter-stimulus-interval 2 sec] in order to optimize coverage of the hemodynamic response function (HRF). Data were smoothed, normalized, realigned and batch-analyzed within SPM 2, using HRF as the basis function.
Results: Cocaine patients pretreated with placebo showed limbic activation by both the "seen" and the "unseen" cocaine cues, whereas patients receiving baclofen pretreatment showed a dramatic and specific blunting of the cue-related limbic response.
Discussion: These data provide the first evidence that the GABA B agonist baclofen may be helpful in blunting the limbic response to very brief, and even to "unseen", drug cues. GABA B agonists may act have anti-relapse potential by targeting drug motivational substrates both outside and within awareness. Supported by: VA Medical Research (VA VISN 4 MIRECC), NIDA (RO1DA10241; RO1DA15149; RO1DA12162; P50; P60), DANA Foundation, and The Alexander Foundation. The authors have no financial interests that would lead to conflict of interest for this work.

11. Characterization of a Novel Brain Penetrant, Orally Available Corticotropin-Releasing Hormone Receptor 1 (CRH-R1) Antagonist for Treatment of Alcoholism

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Background: The CRH system is consistently implicated in stress responses and stress related disorders, but translation into clinical ap-

plication has been hampered by difficulties in developing orally available, brain penetrant compounds targeting CRH-R1 receptors. Here, we describe a novel CRH-R1 antagonist (AAA0206) with advantageous properties for clinical development, and its in vivo acivity in preclinical alcoholism models.
Methods: Locomotor activity and open field were measured in sound attenuated behavioral chambers equipped with an open field (43 x 43 cms) with infra-red beam detectors (Med Associates Inc, St Albans, VT). Elevated plus-maze was as in (Thorsell et al. 2000). % open time and entries ((open arms)/(open arms + time closed arms)*100%)) were used as measures of anxiety like behavior. The total number of entries onto any arm was used as an indicator of general activity. When run under ethanol withdrawal conditions, ethanol (3g/kg) was administered ip 12 hrs prior to the animal being put on the plus-maze. Operant self-administration of 10% alcohol was established using a saccharin fading procedure and daily 30 min fixed ratio (FR) 1 sessions, and self-administration and reinstatement were studied as described (Ciccocioppo et al. 2002).
Results: AAA0206 inhibited 125I-sauvagine binding to rat pituitary membranes with sub-nanomolar affinity. Following oral administration in rat, AAA0206 inhibited 125I-sauvagine binding to cerebellar membranes with an ED50 of app. 1.3mg/kg. Oral bioavailability was 91.1%. Compared to R121919 and SSR125543A, AAA0206 had a markedly reduced volume of distribution and clearance. Neither open field activity nor baseline exploration of an elevated plus-maze were affected by AAA0206 (1-10mg/kg), arguing against a tonic activity of endogenous CRH at the CRH1 receptor. In contrast, AAA0206 fully and dose-dependently reversed the anxiogenic effects of withdrawal 12h after a 3g/kg EtOH dose. Similarly, AAA0206 did not affect basal EtOH self-administration in non-dependent Wistar rats, but blocked excessive self-administration seen following a history of dependence in Wistar rats, and in a genetic model of high alcohol preference, the msP rat. Also, AAA0206 did not affect reinstatement of stress-induced EtOH seeking in non-dependent Wistar rats, but did so both in post-dependent and genetically selected msP animals.
Discussion: These data suggest that central CRH systems are quiescent under baseline conditions, promising an attractive tolerability profile for compounds targeting this system. Furthermore, suppression of ongoing excessive EtOH self-administration and prevention of relapse following abstinence, two key objectives for any novel alcohol dependence treatment, are effectively achieved by the antagonist (AAA0206) in standard preclinical models. In summary, we confirm and extend prior findings showing that up-regulated activity of the CRH system confers susceptibility for excessive self-administration of alcohol, and for relapse into alcohol seeking following abstinence. We then provide the first data to demonstrate the efficacy of a novel, orally available and brain penetrant CRH-R1 antagonist to effectively target this pathology. This antagonist is a promising candidate for treatment of alcohol dependence.

12. Risk Factors for Substance Use Disorders: A 3-Generation Family Study
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Sponsor: Myrna M. Weissman

Background: We studied risk factors for drug and alcohol use disorders in families at high and low risk for depression. These data were gathered as part of an on-going longitudinal study of mood and anxiety disorders in high risk families. This is a unique sample that includes 3 generations, and has been followed for more than twenty years. The sample was originally designed to study the psychiatric and behavioral problems among children of parents with (high-risk) and without (low-risk) Major Depressive Disorder (MDD). Although the original focus of the study was on depressive disorders, we have collected extensive information on the onset, chronicity and severity of drug and alcohol use, abuse and dependence. The on-going study therefore affords a unique opportunity to examine the course of substance use, both within individuals, and across generations, without being hampered by limitations of cross-sectional designs. We present here data on the course of drug and alcohol use disorders in relation to other psychiatric disorders through the 20 year follow-up in the second generation (G2), and the impact of these disorders on the psychopathology and functioning of their offspring (G3).
Methods: The second generation (G2) was interviewed at four waves. The baseline sample (wave 1) consisted of 220 offspring, ages 6-23 years, from 91 families. They were interviewed again approximately 2 (Wave 2; N = 203), 10 (Wave 3; N = 182) and 20 (Wave 4; N = 151) years later. At each wave subjects received a full diagnostic interview based on the Schedule for Affective Disorders-Present and Lifetime Version (adults) or the Kiddie-Schedule for Affective Disorders (children). Their offspring (G3) were assessed from the third wave onwards. All diagnostic assessments were administered by trained doctoral- and master's level mental health professionals who were blind to the clinical status of the probands, and to information obtained at previous waves. Final diagnoses were made based on the best estimate procedure by two experienced clinicians not involved in the interviewing.
Results: Forty (20%) G2 offspring had a lifetime diagnosis of a drug use disorder (DUD), 47 (27%) had an alcohol use disorder (AUD), and 65 (33%) had either (SUD). The mean ages of onset for alcohol and drug use disorders were 21 and 18, respectively, and all but one onset were post-pubertal. Substance dependence was more than three times as likely among offspring at high risk for MDD than those at low risk, but there were were no group differences for substance abuse. When examined by major classes of psychiatric disorders, DUDs and AUDs were both significantly associated with the presence of major depressive disorder (mean age of onset, 19) and disruptive behavior disorders (age 11), but not with any anxiety disorder age 11). Analyses of the third generation data are underway.
Discussion: Substance use results in significant impairment at both the individual and the societal level, and identification of risk factors and patterns will help enable detection for those at greatest risk. Furthermore, we have also begun to supplement these clinical data with measures of brain activity (EEG and functional MRI) and genotyping studies. Integration of these measures with our clinical findings will allow us to most comprehensively address the genetic and neurobiological mechanisms underlying substance use, and to identify potential vulnerability markers for predicting its disorders. This poster will present the initial clinical findings in the second and third generations.

13. Differential Effects of Acute and Chronic MDMA ('Ecstasy') Administration on Neurotransmitter Efflux and Sensory-Evoked Discharge in the Ventral Posterior Medial Thalamus
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Background: MDMA ('Ecstasy') is known to enhance tactile sensory perception, which has been reported to contribute to its abuse liability. To date no published literature exists that addresses the neurophysiological mechanisms underlying MDMA's effects on somatosensation; however MDMA interactions with the central serotonergic and noradrenergic systems via blockade of serotonin and norepinephrine transporter protein function (SERT & NET) are well known. The rat trigeminal somatosensory system has been well defined and receives serotonergic and noradrenergic afferents from the dorsal raphe and locus coeruleus nuclei, respectively. These fibers express SERT and NET, and are particularly vulnerable to MDMA-induced effects. The goal of the present study was to to determine the effects of acute and chronic MDMA exposure on monoamine transmitter efflux and sensory-evoked discharge in the ventral posterior medial (VPM) thalamus, the major thalamic relay along the trigeminal somatosensory pathway.

Methods: We conducted in vivo microdialysis and multi-channel multi-unit recordings in awake and halothane anesthetized male Long Evans Hooded rats, respectively. Additionally, in order to fully characterize the physiological actions of low-dose MDMA (3 mg/kg, i.p.) we measured the drug's acute effects on plasma levels of MDMA and its major metabolite methylenedioxyamphetamine (MDA). All experimental protocols in these studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
Results: First, using microdialysis and HPLC, we found that acute low-dose MDMA administration (3 mg/kg, i.p.) led to a significant increase in 5-HT, but not NE efflux, in the VPM thalamus. In addition, we demonstrated that administration of a challenge injection (3 mg/kg i.p.) following chronic MDMA treatment (3 mg/kg per day; 4 days) elicits both 5-HT and NE efflux in this region. Importantly, extracellular 5-HT levels remain elevated longer in chronically treated animals. Next, we evaluated the potential for MDMA to modulate whisker-evoked discharge of individual thalamic neurons. After surgically implanting stainless steel 8-wire multi-channel electrode bundles, we recorded spike train activity of single cells while activating the ascending whisker pathway using a piezoelectric mechanical stimulator. Using the same dosing regimens as those in the microdialysis studies, we found that both acute and chronic low dose MDMA administration increased the spontaneous firing rate, but reduced both the magnitude and duration of whisker-evoked discharge in individual VPM thalamic neurons of halothane anesthetized rats. In addition, the timecourse of drug action on neuronal firing patterns was consistent with fluctuations in neurotransmitter efflux and drug plasma levels as shown by our microdialysis studies and plasma blood level analysis, respectively.
Discussion: Based on our current results, we propose the working hypothesis that ecstasy alters tactile experiences in human subjects by disruption of normal patterns of signal transmission through somatosensory thalamic relay circuits. This work was supported by NIH NIDA Grant 1 F31 DA018469-01A1 to Melanie Starr.

14. Development of Robust Intravenous Nicotine Self-Administration Behavior in Drug-Naive Squirrel Monkeys with No Experimental History Under Fixed-Ratio and Progressive-Ratio Schedules of Reinforcement

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Nicotine, a psychoactive component of tobacco plays a major role in smoking dependence, but reinforcing effects of nicotine that contribute to smoking dependence have been difficult to demonstrate directly in past controlled laboratory studies with both animals and humans as experimental subjects. Although the ability of nicotine to act as a reinforcer in experimentally naïve rodents has been demonstrated under limited conditions, its ability to act as a reinforcer in experimentally naïve non-human primates is still unclear.
Methods: Naive squirrel monkeys with no previous history were trained to self-administer nicotine at the dose of 10 microg/kg/inj. Sessions were one hour duration with time-out period of 60 sec. Training was made under fixed-ratio schedule of reinforcement and progressive-ratio schedule of reinforcement.
Results: In the present experiments, intravenous nicotine self-administration behavior developed in drug-naive squirrel monkeys with no history of operant behavior training and no setting conditions such as food deprivation. Once self-administration behavior developed, nicotine sustained robust responding under a Fixed-Ratio schedule of reinforcement where 10 lever presses were required to produce each intravenous injection. The behavior was under the control of nicotine injections since lever pressing extinguished when the nicotine solution was changed to saline solution and varying the injection dose of nicotine resulted in a typical inverted U-shaped dose-response curve. Nicotine self-administration behavior also maintained at high rates under a Progressive-Ratio schedule of reinforcement and varying nicotine dose again resulted in an inverted U-shaped dose-response curve.
Discussion: These results demonstrate that nicotine can function as a prototypic drug of abuse serving as a robust reinforcer in squirrel monkeys. This non-human primate represents a valuable animal model for studying the neurobiological basis of nicotine dependence. Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse (NIDA), National Institutes of Health and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003). Research was supported by the Intramural Research Program of the NIDA, NIH, DHHS.

15. Effect of Varenicline on Cue-Provoked Cigarette Craving and Acute Nicotine Withdrawal

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: The efficacy of varenicline tartrate, an $\alpha 4\beta 2$ nicotinic receptor partial agonist, has been recently established for the treatment of nicotine dependence. However, little is known about its potential mechanisms of action in smokers. This purpose of this experiment was to evaluate the effect of varenicline on cue-provoked craving and acute nicotine withdrawal using a randomized, double-blind, placebo-controlled, cross-over design.
Methods: Participants were 40 adult smokers (36 years-old, 21 cigs/day, Fagerstrom Tolerance Questionnaire score of 6) who were not interested in quitting smoking. Following biochemically-confirmed, overnight abstinence (12 hours), participants received either 2 mg varenicline or placebo. Smoking cue-reactivity was assessed 4 hours later. During this procedure, participants were exposed to a cigarette cue and a neutral cue in a pre-assigned sequence of either active-neutral or neutral-active. Each participant followed the same presentation order for both treatment sessions. In the cigarette cue condition, the participant lit and held his/her preferred brand of cigarette and then extinguished it. In the neutral cue condition, the participant held and sharpened a pencil. Each cue exposure lasted one minute. Participants completed questionnaires of craving (Smoking Urges Scale) and nicotine withdrawal symptomatology (Minnesota Nicotine Withdrawal Scale) immediately after each cue exposure and again 5 and 10 minutes later. Varenicline and placebo sessions were separated by a one week wash-out period.
Results: Subjective craving and withdrawal symptom scores were analyzed using repeated measures analysis of variance. The within-subjects factors were treatment (2 mg. varenicline/placebo), cue type (cigarette/neutral), time (immediate post-cue exposure/5 min./10 min.), treatment order (varenicline first/placebo first), and treatment session (session 1/session 2). In contrast to expectations, the treatment x cue type interaction was statistically non-significant for both craving ($p=.70$) and withdrawal ($p=.67$). The primary results were significant main effects of treatment on both craving [$F(1, 36)=30.1$, $p<.001$] and withdrawal [$F(1, 36)=18.4$, $p<.001$]. Treatment with varenicline, as compared to placebo, attenuated the craving response to both cigarette ($p<.001$) and neutral cues ($p<.001$), independent of time, treatment order, and session. Craving scores in response to the cigarette cue were 40.8 ± 2.1 after varenicline and 52.9 ± 2.1 after placebo. Neutral cue craving scores, which were significantly lower

(p<.001), were 34.6±2.0 during varenicline and 45.1±2.0 during placebo. There was a similar effect of treatment on withdrawal scores: cigarette cue (varenicline 5.1±0.3, placebo 7.4±0.3); neutral cue (varenicline 5.3±0.3, placebo 7.3±0.3).
Discussion: Single dose (2 mg.) varenicline does not appear to influence cigarette cue-provoked craving in abstinent smokers. This initial experiment suggests that varenicline may attenuate tonic levels of craving and acute nicotine withdrawal. Future analyses of these data will test for potential gender differences in craving and withdrawal response to varenicline. Funded by Pfizer.

16. NMDA Receptor Deficient Mice Display Altered Synaptic Function and Reward Related Behaviors

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Sponsor: Travel Awardee, sanofi-aventis, 2006

Background: In the present study, NMDA hypomorph mice (NR1 mice), with a 90% reduction in NMDA receptors, were used to examine glutamate-dopamine-ethanol interactions. The antagonistic action of ethanol on NMDA receptors is an important mechanism through which ethanol alters CNS function. Since an elevation of dopamine is implicated in the stimulating and reinforcing properties of ethanol, the dopamine system was evaluated to see if differences in ethanol-related behaviors between wild type and NR1 mice could potentially be related to alterations in the dopaminergic system.
Methods: To evaluate NMDA receptor function in hippocampus (CA1 region) and nucleus accumbens (core region) brain slices, whole-cell patch clamp methods were used. Behavioral tests, including monitoring locomotor activity in response to ethanol (2 g/kg, i.p.) and the dopamine uptake inhibitor GBR-12909 (5 mg/kg, i.p.) and a two-bottle choice drinking paradigm (10-day, 10% ethanol or water) were used. Finally, voltammetry in striatal slices was used to monitor electrically-evoked dopamine release as well as evaluate D2 autoreceptor function with quinpirole (1 nM - 1 μM) application.
Results: Bath application of 100 μM NMDA induced inward currents in all hippocampal and nucleus accumbens neurons recorded from slices prepared from NR1 mice and their wild type controls; these currents reversed completely during NMDA washout and were fully antagonized by the NMDA receptor antagonist APV. However, the amplitudes of these currents were significantly smaller in NR1 mice than those recorded from wild type controls (range: 390-1200 pA in wild type vs. 25-150 pA in NR1 mice), even with longer NMDA applications. In CA1 pyramidal cells, under recording conditions in which GABAergic synaptic transmission was blocked to evaluate synaptic NMDA receptor function, electrical stimulation of glutamatergic afferents evoked inward currents in all cells recorded. Slices were treated with a maximal concentration of the AMPA/kainate receptor antagonist DNQX (20 μM) to determine the percentage of EPSCs that were mediated by NMDA receptors. In slices from wild type mice, the NMDA receptor-mediated component made up 59.8%±3.5% of the area of the EPSCs. In contrast, only 19.0%±2.2% of EPSC area was mediated by NMDA receptors in cells recorded from NR1 mice. In both groups of slices, the remaining EPSCs recorded in the presence of DNQX were completely blocked by APV, indicating that they were NMDA receptor-mediated. These data suggest that both total and synaptic NMDA receptor functions are significantly reduced in NR1 mice. Interestingly, synaptic NMDA receptor function was only reduced by approximately 60-70%, despite a 90% reduction in NMDA receptor binding and in total NMDA receptor function, suggesting that there may be partial compensation at the level of the synapse. Behaviorally, wild type mice showed significant locomotor activation upon ethanol injection while NR1 mice showed marked sedation. NR1 mice also demonstrated reduced preference for drinking ethanol in comparison with wild type mice in the two-bottle choice drinking paradigm. GBR-12909 caused a 10-fold increase in locomotor activity in wild type animals, but had no significant effect in NR1 mice. Using voltammetry in brain slices, we found that electrically-stimulated dopamine release in the NAc core was reduced by 50% in NR1 mice, compared to wild type mice. Additionally, dorsal striatal D2 autoreceptors, which regulate dopamine release, were found to be less sensitive in NR1 mice.
Discussion: These results suggest that reduced NMDA receptor function in NR1 mice leads to disruption of dopamine function which may decrease the stimulating and reinforcing effects of ethanol. Thus, it appears that the NMDA hypomorph mouse is a unique tool with which to evaluate interactions between NMDA receptors, dopamine and ethanol effects.

17. A Double-Blind Study Comparing Varenicline with Bupropion-Sr and Placebo for Smoking Cessation: Results from a Single Center

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Sponsor: Ernest P. Noble

Background: The need for safe and efficacious compounds for smoking cessation is well known. While more than 80% of cigarette smokers express a desire to stop smoking, and approximately 41% try to stop smoking each year, the percentage of smokers achieving and maintaining abstinence for any significant period of time is disappointing at best. Varenicline, a novel α4β2 nicotinic acetylcholine receptor partial agonist, was recently approved in the United States for smoking cessation. The study objective was to assess the efficacy and safety of varenicline for smoking cessation versus bupropion-SR and placebo. This analysis compares data from our center to the multicenter results.
Methods: As part of a larger, randomized, double-blind, parallel group, placebo- and active-treatment-controlled, multicenter, Phase 3 study, we pre-screened > 1,000 adult smokers recruited via radio, television, and newspaper advertising at this center. From this sample 78 generally healthy smokers (≥10 cigarettes/day), with fewer than 3 months abstinence within the past year, received study treatment. Participants received brief counseling and were randomly assigned to receive varenicline titrated to 1 mg/bid (n=28), bupropion-SR titrated to 150 mg/bid (n=24), or placebo (n=26) for 12 weeks, followed by 40 weeks of post-treatment follow-up. The primary endpoint was exhaled carbon monoxide-confirmed 4-week continuous abstinence rate for weeks 9-12 and the key secondary endpoints were continuous abstinence rate for weeks 9-24 and weeks 9-52. Endpoints are presented for this single center in comparison to the multicenter results. The trial was not powered to analyze single-center data.
Results: The primary endpoint of 4-week continuous abstinence rate for weeks 9-12 was greater for varenicline (single center: 64.3%; multicenter: 44.4%) than for bupropion-SR (single center: 50.0%; multicenter: 29.5%) or placebo (single center: 26.9%; multicenter: 17.7%). Multicenter results for 4-week continuous abstinence rate for weeks 9-12 were significantly better for varenicline than for bupropion-SR (p<0.001) and placebo (p<0.001). Post-treatment continuous abstinence rates at Week 24 in the varenicline group were higher (single center: 50%; multicenter: 29.8%) compared with bupropion-SR (single center: 29.2%; multicenter: 20.7%) and placebo (single center: 19.2%; multicenter: 10.5%). This pattern was still evident at Week 52, with more varenicline-treated patients demonstrating continuous abstinence (single center: 39.3%; multicenter: 22.1%) than those treated with bupropion-SR (single center: 20.8%; multicenter: 16.4%) or placebo (single center: 19.2%; multicenter: 8.4%). Multicenter results showed that varenicline produced significantly better continuous abstinence rates at Weeks 24 and 52 compared with placebo p<0.001) and at Week 24 compared with bupropion-SR (p=0.006). The most commmon adverse events overall in the active

treatment groups were nausea (28.1% of the varenicline group) and insomnia (21.9% of the bupropion-SR group). Varenicline was generally well tolerated.
Discussion: Overall, varenicline was significantly more efficacious than placebo throughout the entire 52-week study and more efficacious than bupropion-SR at Weeks 12 and 24. These single center results were consistent with the multicenter study and demonstrate the successful continuous abstinence rates achievable in a single center setting.

18. ADH and ALDH1A Alleles are Associated with Alcohol Dependence in Trinidad and Tobago
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Background: Trinidad is the southernmost island of the Lesser Antilles in the Caribbean. The population of Trinidad is multi-ethnic but mainly composed of people of East Indian (Indo-Trinidadians [TT]) and African (Afro-TT) ancestry. The prevalence of alcoholism in Trinidad and Tobago is higher than in the US and also among patients of East Indian ancestry when compared to Africans (prevalence rates of 47% and 33% respectively). Since variants in alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)genes differ between ethnic the present study sought to determine whether an association exists between ADH1C, ADH1B, and ALDH1A genotypes, alcohol dependence, drinking history, and liver function tests in the two major ethnic groups of Trinidad and Tobago (TT).
Methods: One hundred and forty-five alcoholic dependent individuals of both East Indian (Indo-TT) and African (Afro-TT) ancestry and 108 controls matched by age, sex, education were assessed using the SSAGA. Serum levels of (ALT, AST,ALP,LDH, GGT) as well as presence of HIV, hep B and C virus antibody were determined. DNA was isolated from leukocytes and ADH1C and ADH1B2 ADH1B3,ALDH1A polymorphisms determined. The relevant portions of the ADH and ALDH1A loci were amplified using the polymerase chain reaction followed by hybridization with allele specific radiolabeled oligonucleotide probes.
Results: There was a significant difference in the distribution of ADH1C allele polymorphisms between the ethnic groups ($P<0.0001$). Forty-three percent of the Indo-TT were found to have one ADH1C*2 allele and 23% of Afro-TT. The ADH1C*2 allele was significantly associated with alcohol dependence and significantly elevated levels of ALP ($p<0.02$) and GGT ($p<0.02$). Twenty-four participants (10%) possessed the ALDH1A1*1/*2 genotype (frequency=.05), four were Afro-TT and 20 were Indo-TT. Indo-TT participants with at least one ALDH1A1*2 allele were more likely to have a lifetime diagnosis of DSM-III-R alcohol dependence ($p<0.002$) and significantly higher levels of current alcohol consumption ($p<0.05$). Twenty-eight of the Afro-TT participants (41%) and one Indo-TT (> 1%) had at least one ADH1B*3 allele and 3 individuals had an ADH1B*2 allele. Individuals with at least one ADH1B*3 allele were found to: be significantly less likely to be alcohol dependent ($p<0.002$), and had lower alcohol consumption levels ($p<0.01$). Among those participants who were alcohol dependent, ADH1B*3 was associated with significantly higher levels of ALT ($p<0.05$).
Discussion: These findings suggest that differences in the distribution of ADH and ALDH1A alleles may in part explain the disparity in prevalence of alcohol dependence and alcohol related morbidity and mortality between the two ethnic groups of Trinidad and Tobago (Supported by AA014370).

19. Effects of Chronic Naltrexone on Ethanol and Sucrose Reinforced Responding in Ethanol-Dependent Rats
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Background: Naltrexone is an opiate-receptor antagonist that has been shown to decrease alcohol intake in animal models and to be useful clinically for attenuating alcohol craving and postponing relapse. We have previously observed that, in nondependent rats, chronic naltrexone treatment nonselectively decreased intake of ethanol and sucrose during operant sessions. However, when these subjects were subsequently tested for reinforcer-seeking after naltrexone treatment ended (i.e., no naltrexone on board), only ethanol-seeking was decreased while sucrose-seeking was unaffected.
Methods: The present experiment examined rats made ethanol-dependent in vapor/inhalation chambers, and cycled through periods of dependence and withdrawal/recovery. Dependence periods were 14 days long with 14 hrs of vapor exposure/day (yielding blood ethanol concentrations averaging 226 mg%), followed by 7 days of recovery with no ethanol access. Finally, 4 days of daily operant sessions with either 10% ethanol (n=7) or 2% sucrose (n=8) as the reinforcer were conducted with a single, non-reinforced extinction session on day 5. Three of these cycles were conducted with no treatment in cycle 1, saline injections preceding operant sessions in cycle 2, and naltrexone (-30min; 0.3mg/kg) treatment preceding operant sessions in cycle 3. There was then one week of operant sessions/naltrexone treatments and a final reinforcer-seeking test with no naltrexone on board. This protocol was intended to include sessions comparable to the previous experiment in nondependent subjects, and to model the clinical situation where the individual has experienced repeated dependence/withdrawal cycles and then seeks treatment.
Results: As in the nondependent subjects, naltrexone decreased intake of both sucrose and ethanol (average saline: 0.92 g/kg; naltrexone: 0.53 g/kg) during all operant sessions. Naltrexone also attenuated reinforcer-seeking in both groups when the drug was on board. When subjects were tested after naltrexone treatment concluded, ethanol-seeking actually rebounded to nontreatment-like levels while sucrose-seeking remained significantly lower.
Discussion: In the nondependent subjects, the decrease in extinction responding (seeking) was interpreted as a change in incentive motivation resulting from the reinforcer devaluation caused by drug/reinforcer pairings, which was selective for the ethanol group. Those findings suggested that in the early stages of "problem drinking" in humans, naltrexone could be a good intervention for this level of alcohol abuse and relapse prevention. The present findings, however, indicate that in ethanol-dependent subjects, naltrexone reduced the intake and motivation to seek drug (ethanol) and nondrug (sucrose) reinforcers when treatment was on board but failed to sustain the attenuation of ethanol-reinforced responding following the discontinuation of treatment. Alterations in the opiate system resulting from repeated cycles of ethanol dependence and withdrawal may account for the differential effects of naltrexone in the nondependent and dependent animals. All procedures in accordance with NIH Guide; Supported by NIAAA AA013860

20. Effects of Postnatal Methylphenidate Exposure on Reward-Mediated Behavior in Early Adulthood
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Background: Methylphenidate (Ritalin) usage in preschool aged children (2- to 5-year-olds) has become increasingly common over the last decade. The increased use of methylphenidate is of potential concern because there are few studies examining the long-term effects of stimulants in this age group. Developmental studies in rodents suggest that early exposure to methylphenidate may alter later responsiveness to drugs of abuse and cause persistent changes in neuronal functioning. Previous research examining early methylphenidate exposure has not produced a consistent pattern of results, with some studies showing that methylphenidate exposure increases later drug responsiveness while other studies report the opposite effect. An interesting pattern emerging from these studies is that exposing rats to methylphenidate during the adolescent period appears to increase

later drug responsiveness, while exposing rats to methylphenidate during the earlier preadolescent period may reduce responsiveness to later psychostimulant administration. Preliminary data gathered in our laboratory indicates that methylphenidate exposure during the preweanling period (i.e., on postnatal day (PD) 11 to PD 20) enhances later responsiveness to rewarding stimuli. These findings suggest that methylphenidate exposure during this developmental period may increase later drug vulnerability.
Methods: The goal of the present study was to further examine the effects of methylphenidate exposure during the preweanling period. To this end, we examined the acquisition, extinction, and reinstatement of morphine- and cocaine-induced conditioned place preference (CPP) in 60-day-old rats that had been exposed to methylphenidate (2 or 5 mg/kg) or saline on PD 11–PD 20. A 10-day CPP procedure was used to assess the initial acquisition of morphine and cocaine-induced CPP, which included one preconditioning day, eight conditioning days (consisting of alternating daily injections of saline or drug), and one test day. After conditioning and the initial CPP test, rats were given extinction training where they received daily injections of saline before alternating 30-min placements in the black and white compartments. Extinction conditioning lasted eight days and was followed by a test day. Following this test day, rats were given a priming dose of morphine or cocaine 30 min before being tested for reinstatement of CPP. In addition to the CPP experiments, we also assessed the effects of early methylphenidate exposure on sucrose-reinforced lever pressing using a progressive ratio schedule.
Results: Early methylphenidate treatment was found to have no effect on the acquisition of morphine- or cocaine-induced CPP. During the extinction phase, however, rats previously exposed to methylphenidate (5 mg/kg) spent more time in the morphine-paired chamber than did rats previously exposed to saline during the preweanling period. Early methylphenidate exposure also increased the amount of time spent in the cocaine- and morphine-paired chamber during the reinstatement phase. In Experiment 2, early methylphenidate exposure (2 and 5 mg/kg) enhanced the reinforcing effects of sucrose, because rats exposed to methylphenidate on PD 11–PD 20 had greater breakpoints (defined as the number of reinforcements received on the progressive ratio schedule) than saline-exposed rats.
Discussion: The present data suggest that early exposure to methylphenidate could lead to greater vulnerability to drug use and relapse in adulthood. Moreover, because early methylphenidate exposure altered sucrose-reinforced responding, as well as psychostimulant-induced CPP, it appears that the neural changes resulting from methylphenidate exposure have a more generalized effect on positively reinforced behaviors, perhaps by altering neural reward mechanisms.

21. Do Adolescent Rats Take Drugs More Because They are Less Aversive?

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Background: Drug use that begins during adolescence is more likely to progress to addiction than use which begins later in life. Age-related differences in the reinforcing and/or aversive properties of the drug experience could contribute to this vulnerability. However, drug use is also influenced by individual susceptibility and environmental exposure. The purpose of the present study was to evaluate the interaction of individual differences and developmental stage on cocaine consumption in rats. We investigated the relative contribution of individual differences in novelty seeking and anxiety on the aversive effects of cocaine and on voluntary cocaine consumption. The underlying hypothesis was that adolescents would find cocaine less aversive and consume more in a voluntary intake paradigm, and that high novelty seeking and low anxiety would predict cocaine consumption.
Methods: Rats of postnatal age 28 and 65 days were pre-screened by evaluating the locomotor response to novelty and behavior on the elevated plus maze. They were then assigned to either a cocaine conditioned taste aversion (CTA) trial to assess the aversive effects of cocaine or a voluntary cocaine drinking paradigm. To assess CTA, rats were water deprived for 24 hours, then given 0.2% saccharin to drink followed 15 minutes later by saline, cocaine (10, 20 or 40 mg/kg) or LiCl (19 or 76 mg/kg) and returned to the home cage. Twenty four hours later, they were given access to a choice of saccharin or water and fluid intake was assessed. For voluntary cocaine consumption, rats were given a cocaine/saccharin solution (500 mg/L of each) as the sole source of fluid during a 5 hour drinking session for 3 days followed by 3 days of a choice of saccharin or cocaine/saccharin. Intake of each was quantitated each day and results averaged. Means were analyzed by 3-way ANOVA followed by nested lower level ANOVAs and posthoc tests. Potential correlations between novelty locomotion and plus maze performance, and cocaine CTA and voluntary consumption were tested by linear regression.
Results: Relative to adults, adolescent rats drank more cocaine but not more saccharin in the two-bottle choice test and were also significantly less sensitive to the aversive effects of both cocaine and lithium in the CTA task. These results suggest that reduced aversive effects of cocaine in adolescents may contribute to enhanced cocaine intake. Linear regression analysis showed that novelty-seeking correlated with cocaine drinking in adolescents while anxiety was associated with adult cocaine drinking. Novelty locomotion was positively correlated with cocaine intake and negatively correlated with saccharin intake in adolescents. In adults, novelty seeking was not correlated with cocaine intake but was positively correlated with saccharin intake. Anxiety was inversely correlated with cocaine consumption in adults. Neither novelty nor anxiety predicted responses to CTA.
Discussion: One potential interpretation of these data is that adolescents are relatively insensitive to the aversive experiences caused by drug taking, so other characteristics, especially novelty/sensation seeking are more important predictors of drug taking. However, in adults, the aversive properties of drug taking (e.g., the bitter taste of cocaine) restrains drug consumption. These findings suggest that different tempermental characteristics support experimentation and progression of drug taking in adolescence and adulthood, an hypothesis which should be tested using rigorous operant paradigms which better quantitate the reinforcing effects of drugs of abuse. All experiments were approved by the institutional IACUC. Supported by DA09079 and DA019114

22. Sustained Release Naltrexone Modulates Brain Response to Drug Cues in Heroin Addicts

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Sponsor: Anna Rose Childress

Background: Naltrexone is a pure opiate μ-receptor antagonist that has been shown to be an effective treatment of alcohol and heroin dependence at the pharmacological and behavioral levels (Cornish et al., 1997). Functional brain imaging data indicate that addiction is characterized by abnormal balance of behavioral control and reward seeking (Breiter and Rosen, 1999; Gottfried et al., 2003). The goal of this study is to demonstrate with BOLD fMRI, the effects of Naltrexone on the brain correlates of behavioral control and reward in treatment seeking heroin addicts.
Methods: In an ongoing study (N = 5), recently abstinent heroin addicts underwent fMRI at 3 Tesla, during exposure to multiple drug-related and neutral visual cues 2 ("brief") and 30 ("long") seconds in duration, immediately before ("PRE NTX" condition) their first Naltrexone injection (228 mg effective dose, Depotrex®, Biotek) and 2 weeks thereafter ("ON NTX" condition) (Comer et al., 2006). The brief cues task presentation is fast event-related and random. Forty-

eight different "Drug", "Neutral" stimuli are presented twice, 2 seconds at a time. "Drug" stimuli are images of heroin preparation and injection, "Neutral" stimuli are images of house and office work, matched for luminosity and semantic content to the drug stimuli. The "long" cues task is a block design experiment consisting of 30-second long video clips from a documentary about street heroin users ("Drug") and a film about home improvement ("Neutral"), presented in a pseudo-random fashion and separated by 6-second intervals. fMRI data are converted into 3D ANALYZE files, time-slice and motion corrected, co-registered to own T1 image, normalized to standard coordinate system (MNI) and smoothed with an 8-mm FWHM Gaussian kernel. Individual-level contrasts of the preprocessed data are generated with the General Linear Model. T-tests and ANOVA are used to generate the following group-level contrasts: PRE NTX [Drug vs Neutral]; ON NTX [Drug vs Neutral]; ON NTX [Drug] vs PRE NTX [Drug], for each task.
Results: Preliminary analysis of the brief cues data shows a response to Drug cues in the Orbitofrontal (OFC) and Medial Frontal (MFC) cortex, bilateral amygdalae, insulae, striatum, premotor, posterior parietal and caudal anterior cingulate (ACC) cortices. ON NTX, there is a trend for reduced amygdala, OFC and premotor response and increased MFC and inferior frontal cortex response (BA 11,10,45,46) to Drug cues. Preliminary analysis of the "long" cues data shows a response pattern similar to the brief cues data, with smaller effect size.
Discussion: Depo-naltrexone administration modulates the brain response to heroin cues in abstinent heroin addicts. The response in the cortico-limbic motivation and reward system is reduced while the response in the prefrontal associative and behavioral regulation network is increased. A limitations of these preliminary data is the fact that the neurobiological effect of depo-naltrexone could be confounded by the less specific effects of abstinence. In absence of a placebo group, this issue could be addressed by studying patients when and if they discontinue depo-naltrexone treatment. If confirmed in a larger sample, these data could form the basis of fMRI-guided treatment planning and monitoring of depo-naltrexone therapy in heroin dependent patients.

23. Contribution of Extrasynaptic Glutamate to Preclinical Models of Cocaine Addiction: Repeated N-Acetylcysteine Administration Selectively Blocks Plasticity-Dependent Behaviors

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Background: Drug-induced plasticity has been proposed to contribute to the persistent, chronic-relapsing nature of cocaine addiction. Animal models dependent, in part, on plasticity include behavioral sensitization, escalation of drug intake during extended access self-administration, and drug-primed reinstatement. Cocaine-evoked neurochemical plasticity that has been implicated in at least some of these behaviors includes a persistent reduction in basal glutamate levels leading to an augmented response following a cocaine challenge.
Methods: The present study utilized the cysteine prodrug N-acetylcysteine (0-60 mg/kg, IP; NAC) to manipulate the activity of cystine-glutamate antiporters in an attempt to prevent behavioral and neurochemical plasticity as described below. Behavioral Sensitization: Rats received seven daily injections of cocaine (15-30 mg/kg, IP); locomotor activity was assessed on days 1 and 7 for 2 hr following the cocaine injections. Twenty one days later, rats were challenged with cocaine (15 mg/kg, IP) and locomotor activity was assessed for 2 hr. Access-dependent Escalation of Cocaine Intake: Long-access rats (LA) were permitted to self-administer cocaine (1.0 mg/kg/inf, IV) for six hrs over eleven daily sessions. Intake on day 11 was compared to day 1 to determine escalation of intake. Cocaine-Primed Reinstatement: Rats were trained to self administer cocaine under short access conditions (2 hrs/day; ShA) for 11 daily sessions. Responding was extinguished by discontinuing cocaine infusions following lever presses. Responding was reinstated by challenging rats with a cocaine primer (10 mg/kg, IP). During reinstatement, rats also underwent microdialysis testing to permit sampling of glutamate in the nucleus accumbens.
Results: Behavioral Sensitization: Rats receiving daily cocaine injections exhibited behavioral sensitization when tested 21 days following the last drug treatment. NAC pretreatment did not alter acute cocaine-induced locomotor activity, but prevented behavioral sensitization. Access-dependent Escalation of Cocaine Intake: LA rats exhibited a significant increase in daily cocaine intake. Rats pretreated with NAC exhibited normal levels of intake on day 1, however, these rats failed to exhibit an increase across sessions. Cocaine-Primed Reinstatement: ShA rats exhibited stable levels of intake during self-administration that was returned to controls levels following extinction training. A cocaine primer significantly elevated lever responding. These rats also displayed a persistent reduction in basal glutamate levels and an augmented response following the cocaine challenge. Rats pretreated with NAC exhibited equivalent levels of cocaine intake during self-administration training; however, responding on the first extinction session and following the cocaine prime was significantly reduced relative to ShA controls. NAC pretreatment also prevented changes in glutamate that were obtained in ShA controls.
Discussion: Collectively, these studies reveal that NAC blocks behavioral and neurochemical plasticity produced by repeated cocaine including behavioral sensitization, escalation of drug intake during extended access self-administration, drug-primed reinstatement, and altered basal and cocaine-evoked glutamate without impacting behavior produced by acute cocaine administration. The selective effects of NAC on plasticity-dependent behaviors further supports the hypothesis that extrasynaptic glutamate maintained by nonvesicular release mechanisms contribute to cocaine-induced plasticity that is pathogenic for compulsive drug-seeking behavior. Supported by DA017328

24. The Effects of Topiramate and Zonisamide in Combination with Alcohol on Spatial Memory

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Sponsor: Conan Kornetsky

Background: Topiramate and zonisamide are novel anticonvulsants that reduce ethanol consumption when administered to either mice or rats, and that may be of use as medications for the treatment of alcoholism. The objective of the present study was to determine whether the administration of either topiramate or zonisamide, either alone or in combination with ethanol, would result in the alteration of spatial reference memory as assessed by a Morris Water Maze task.
Methods: Longs Evans rats were treated with topiramate (50 mg/kg IP), zonisamide (50 mg/kg IP), or vehicle, in combination with either ethanol (1.2 gm/kg IP) or saline. Animals were trained in the Morris Water Maze for four days prior to testing. The escape platform was kept in a fixed position during both training and testing. Animals were tested in the Morris Water Maze one-hour after the administration of either anticonvulsants or vehicle. The locomotor activity of animals was assessed for 5 minutes immediately prior to testing in the Morris Water Maze.
Results: The dose of ethanol selected for use in this experiment did not significantly alter latency for escape in the Morris Water Maze, when this agent was administered alone. Mean escape latency was significantly greater for the topiramate/ethanol treatment group than for either the vehicle/saline or vehicle/ethanol groups. Although the mean latency value for the topiramate/saline group was larger than were the values obtained for the control group there was great variability in these values amongst individual animals. Latency values for the zonisamide/ethanol group, although modestly elevated, did not differ significantly from values for the vehicle/saline group. Measures

of locomotor activity, including distance traveled and ambulatory counts, did not differ significantly amongst the treatment groups.
Discussion: Topiramate, when administered with alcohol, may impair spatial reference memory at doses of these agents that do not decrease locomotor activity. When administered alone, at the dose used here, topiramate may produce memory impairment in only a subset of animals. Zonisamide may not have the same potential, as does topiramate for altering spatial memory when administered either alone or with ethanol.

25. Relation Between Self-Reported Peripheral Neuropathy and Brain Morphology and Function in HIV Infection with Alcoholism Comorbidity

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Background: Peripheral neuropathy can be painful, debilitating, and cause compromise in quality of life and productivity. It is estimated that 30% of HIV-infected patients are diagnosed with some form of peripheral neuropathy and that nearly 100% of AIDS cases have evidence of peripheral neuropathy at autopsy. Similarly, peripheral neuropathy is the most common neurological complication in alcoholism, and alcohol use disorder (AUD) comorbidity is highly prevalent in HIV infection. Although both HIV infection and AUD can produce peripheral neuropathy, they may do so by different mechanisms. Both disorders also are characterized by central nervous system (CNS) injury, but the relations between CNS and peripheral nervous system injury in HIV infection, alcoholism, and their interaction have not been investigated. As an initial attempt to identify factors associated with or predictive of peripheral neuropathy, we describe brain structural, neuropsychological, and disease variables in HIV-infected patients (HIV) with and without comorbidity for alcoholism (HIV+ALC) and with or without self-reported peripheral neuropathy (PN).
Methods: The study groups comprised 41 HIV patients (9 with and 32 without self-reported PN) and 52 HIV+ALC (13 with and 38 without self-reported PN). Participants were interviewed by SCID, received cognitive and motor testing and hematological study, and were examined with conventional MRI and diffusion tensor imaging. No patient was clinically demented. Group comparisons for neuropsychological and neuroimaging measures were based on standardized Z-scores adjusted for normal age measured in healthy controls, recruited and examined concurrently with the same test and MRI protocols as the patients.
Results: The incidence of PN was similar in the two HIV groups (χ^2=.096, p=.76). All patients were rated at least 80 on the Karnofsky scale, and >90% were rated 90 or 100; however, 61% of PN patients had scores of 90 and 30% had scores of 100, whereas there proportions were reversed in the non-PN patients (χ^2=7.48, p=.024). Patients with PN had a higher incidence of history of an AIDS-defining event (χ^2=12.38, p=.0004), but not of hepatitis C (χ^2=2.38, p=.123), than those without PN. The PN group had poorer ataxia and grooved pegboard scores, smaller callosal genu area, larger ventricular volume (especially in the frontal horns) than the non-PN group, irrespective of alcoholism comorbidity. The comorbid group with PN consumed more alcohol over their lifetime than any other group, had prolonged time to initiate a response (but not to move between targets) in a two-choice test of attention, and disproportionately high diffusivity in the corpus callosum genu and body and low FA in the callosal body. Although the HIV+ALC group had significantly lower Global Assessment of Functioning and CD4 cell counts than the HIV only group, these factors were not related to PN. Neither body mass index, viral load, nor white matter hyperintensity volume distinguished the groups.
Discussion: Incidence of self-reported peripheral neuropathy was associated with frontally-distributed ventricular enlargement and callosal thinning, compromised upper and lower limb motor control, and AIDS in HIV infection irrespective of alcoholism comorbidity. HIV with alcoholism and PN was associated with slowed response time and callosal microstructural compromise. Whether these indices of CNS health contribute to peripheral neuropathy or have a causal relation remains unknown, but their co-occurrence may have implications for treatment regime and responsivity. Supported by AA 12999, AA12388, AA10723, AA05965

26. The Effect of Cycloserine on Smoking Cue-Reactivity and Smoking Behavior in Nicotine Dependent Smokers

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Sponsor: Travel Awardee, NIMH, 2006

Background: Cycloserine (Seromycin®), a broad-spectrum antibiotic used to treat TB, functions primarily as a selective partial NMDA agonist. The activation of the NMDA receptor is important for inducing long-term potentiation, an important neuronal substrate of learning. It is speculated that cycloserine improves cognitive functioning by enhancing the efficacy of glutamatergic transmission via strengthening of NMDA receptors during learning processes. Several studies have demonstrated that the NMDA receptor is critically involved with extinction (Walker et al., 2002; Ledgerwood et al., 2003; Ressler et al., 2004; Hoffman et al., 2006). The results of these studies demonstrate that cycloserine facilitates the effects of exposure therapy and may hold promise when combined with cue exposure and extinction for smoking. The present study examines the effect of cycloserine compared to placebo when combined with two laboratory cue exposure training sessions on smoking behavior in nicotine dependent smokers. We hypothesize that cycloserine, relative to placebo, will facilitate extinction of smoking cue reactivity and lead to greater reductions in smoking as measured by urinary cotinine, alveolar carbon monoxide (CO), and cue-exposure urge-to-smoke scores in nicotine dependent smokers.
Methods: A total of 20 smokers will enroll in this four week between groups, double-blind, placebo-controlled study. Subjects receive two five hour test days consisting of a series of six cue exposure sessions combined with cognitive-behavioral therapy for smoking cessation separated by two weeks, with each test day followed by three follow-up sessions: 24 hrs, 48 hrs, and 1-week. Urinary cotinine and CO levels are measured at each follow-up session, while urge-to-smoke is measured on a 1 ("no urge") to 10 ("extreme urge") Likert scale during each of six cue exposure sessions on test days. Subjects are randomly assigned to receive either 50 mg cycloserine or placebo.
Results: Data collection is currently in the early stage. Baseline average urinary cotinine level across participants (n = 5) is 1908 ng/mL (SD = 661) while average urinary cotinine level across the three follow-up phases beyond test day #1 and #2 is 1171 ng/mL (SD = 570) and 1315 ng/mL (SD = 631). Baseline average CO level across participants is 15.6 ppm (SD = 6.4) while average CO level across the three follow-up phases beyond test day #1 and #2 is 11.8 ppm (SD = 7.2), and 13.9 ppm (SD = 6.3). Participant variability suggests a potential effect of the experimental intervention as measured by carbon monoxide level. At follow-up, 60% of participants (n = 3) exhibited CO levels consistently below their baseline values, beginning with an average CO level of 15.3 ppm at baseline and decreasing to an average CO level of 9.1 ppm across follow-up phases. Remaining participants (40%) failed to show CO change compared to their baseline levels across time. On test day #1, 80% of participants started with a combined baseline average of 5.8 on the urge to smoke scale and decreased across the five remaining phases to an average of 2.8 by the sixth cue exposure session. On test day #2, all participants started with a combined average of 8.7 on the urge to smoke scale and decreased to an average of 3.4.

Discussion: While we are unable to break the blind in this study, preliminary examination of the data exhibit promising variability, indicating that several participants in our current sample responded to the intervention based on CO level, while remaining participants exhibited no CO change compared to baseline levels. All participants exhibited decreased urinary cotinine levels at follow-up compared to baseline levels and all decreased smoking cue-reactivity based on the urge to smoke scale by test day #2, indicating that smoking cue exposure led to a reduction in the urge to smoke.

27. Low Frequency Oscillations in Reaction Time and Heart Rate Variability: Preliminary Results in Children with Attention Deficit Hyperactivity Disorder and Pervasive Developmental Disorders

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Sponsor: F. Xavier Castellanos

Background: The amplitude of low frequency oscillations (LFO) (< 0.1 Hz) in reaction time (RT) is significantly higher in children with attention-deficit hyperactivity disorder (ADHD) compared to controls (Castellanos, 2005; Johnson, 2006). LFO spectral power in heart rate variability is inversely related to vigilance (Egelund, 1982, Hyde, 1997), and is greater in children with ADHD and those with Pervasive Developmental Disorders (PDD) with ADHD-related symptoms (Beauchaine, 2000; Borger & Van der Meere, 2000; Althaus, 1998). Similar LFO of regional cerebral blood flow have been used to map functional neuronal connectivity (Biswal, 1995; Lowe, 1998; Hampson, 2002,), and are associated with "default mode" functional brain networks observed during rest in healthy adults (Raichle, 2001; Fransson, 2005). Default mode activity is related to inter-trial variability (Weissman, 2006), and its physiological suppression during cognitive performance in healthy subjects has not been observed in individuals with autism (Kennedy, 2006). LFO are present in basal ganglia output neurons in rats during wakeful baseline, and are exquisitely modulated by dopaminergic agonists (Ruskin, 2001). In children with ADHD, such RT fluctuations are also normalized by methylphenidate (Castellanos, 2005). This study aims to measure LFO in RT and heart rate variability (HRV) in children with ADHD, PDD and controls, to characterize their modulation from rest by performance of a cognitive task, and to determine their relation to lapses in attention, as measured by errors.
Methods: Data were continuously recorded during a 15 min Eriksen flanker task, preceded and followed by 3 min rest periods. The main performance variables were task accuracy (percent correct) and RT LFO; the main physiological variables were HRV LFO during task and rest periods. LFO power (.02 to .99 Hz) and non-LFO (.10 and .16 Hz) power were computed by Morlet wavelet and Fast Fourier Transform.
Results: A total of 49 subjects were analyzed, including 24 with ADHD (all males, mean age 12 ± 2 years), eight with PDD (7 males, 12 ± 2), and 17 controls (8 males, 12 ± 3). Preliminary results in the entire sample show that a) RT LFO amplitude is significantly higher than RT non-LFO ($p< 0.001$), b) RT LFO correlates significantly with number of errors ($r=0.37$, $p<.05$ and $r= 0.69$, $p<.01$ for directional errors and omissions, respectively), c) HRV LFO is significantly lower during task compared to post-task baseline ($F(1,42)=20.0$ $p<0.0005$), d) HRV LFO recorded during task performance is inversely correlated with the number of correct responses ($r=-0.30$, $p<.05$). In analyses of diagnostic groups, patients showed greater RT LFO and HRV LFO amplitudes than controls, although these differences did not yet reach statistical significance. Data collection is ongoing.
Discussion: These preliminary results demonstrate the simultaneous presence of LFO in both physiological and performance data, and support the hypothesis that a higher level of physiological LFO during a task predicts poorer cognitive performance. LFO in RT and HRV may represent a quantitative endophenotype linking the dynamic interplay of underlying brain functional networks to attentional lapses in ADHD and related disorders. Ongoing studies are exploring the relationships between LFO in RT, HRV, BOLD and psychopathological symptoms in conditions such as ADHD and PDD.

28. Developmental Aspects of Entrained vs. Free-Running Circadian Rhythms

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Background: We have hypothesized that a function of endogenous melatonin production in humans is to augment entrainment of the circadian pacemaker by the light/dark cycle. Some individuals, in whom the retinal-hypothalamic pathway is not able to convey photic information to the circadian pacemaker, have rhythms that free-run with a period typically greater than 24 hours. These individuals are likely to benefit from an external melatonin zeitgeber (time cue) that acts as a signal for "nighttime darkness" and can synchronize (entrain) their circadian rhythms to the 24-hour day/night cycle.
Methods: Twelve blind individuals with no conscious light perception, ages 3 to 20, have been studied to date. Some of these individuals free-run and consequently suffer from recurrent sleep disruption or difficulty staying alert during desired wake hours. The melatonin onset (MO) was used as a marker for circadian phase position. Circadian phase assessments were determined from saliva and/or plasma samples, collected on at least three occasions and assayed for melatonin. Most subjects lived locally and were studied on the General Clinical Research Center at Oregon Health & Science University (OHSU). Other subjects who lived across the country collected saliva samples at home every two hours from waketime until bedtime. Waketime MO phase assessments were based on either an actual MO or an estimated MO, calculated by subtracting the known duration of an individual's melatonin profile from the melatonin offset. Phase assessments revealed either entrainment (an MO occurring at the same time on each sampling occasion) or free-running circadian rhythms (an MO occurring at a different, usually later, time on each sampling occasion). Subjects could then be categorized as either entrained or free-running.
Results: Similar to totally blind adults, we can preliminarily report that approximately half of blind children and adolescents are naturally entrained and half are free-running. In this cross-sectional study comprising four cells (pre- and post-pubertal girls and boys), one of two pre-pubertal girls was entrained while the other was free-running, whereas one of two post-pubertal girls was entrained while the other was free-running. However, all five pre-pubertal boys were entrained and all three post-pubertal boys were free-running.
Discussion: All blind people studied to date in our laboratory are affected by unknown weak zeitgebers. About half of this population free-runs, yet nevertheless remains somewhat sensitive to as-yet-unidentified weak zeitgebers and relatively coordinates to them. The other half of the totally blind population appears to be sufficiently sensitive to these weak zeitgebers so as to naturally entrain to them. This is the first report on a gender-specific difference in the ratio of free-running vs. naturally entrained totally blind people related to (reproductive) development. One of us (AJL) has also proposed that, in addition to the function of endogenous melatonin hypothesized above, third-trimester and early infancy may be a developmental stage in which an external zeitgeber (maternal melatonin transferred via the placenta or breast milk) may help synchronization (to the mother's endogenous circadian pacemaker, provided ambient night time light exposure does not suppress her pineal melatonin production) until the (sighted) infant can directly entrain to the light/dark cycle at about three months of age. This work was supported by PHS Grants R01

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29. Atomoxetine for Children and Adolescents with ADHD and Reading Disorders

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Sponsor: Mauricio Tohen

Background: Atomoxetine, a selective norepinephrine reuptake inhibitor, is a non-stimulant medication approved for treating ADHD in children, adolescents, and adults. Common developmental disabilities of school-age children include ADHD and learning disabilities with prevalence rates of 3-7% and 5-17%, respectively (Goldman, et.al, 1998; Shaywitz, et.al. 1994). Of children with ADHD, it is estimated that 20% will have comorbid dyslexia (Barkley, 2002). Previous atomoxetine studies have demonstrated reduction in ADHD symptoms in the context of comorbid disorders. It is hypothesized that atomoxetine treatment of ADHD+RD could have positive benefits in reducing ADHD symptoms and may have impact on reading performance.
Methods: In this open-label, parallel-design pilot study, patients aged 10 to 16 years with ADHD (n=20) and ADHD+RD (n=36) received atomoxetine for approximately 16 weeks. The ADHD Rating Scale (ADHD RS), the primary efficacy measure and secondary measures of Reading and Spelling Subtests of the Kaufman Test of Educational Achievement (K-TEA), the Working Memory Test Battery for Children (WMTB-C), and Life Participation Scale were evaluated.
Results: Mean change from baseline to endpoint analyses revealed significant improvement for both the ADHD and ADHD+RD groups on the ADHD RS Total score and subscores and LPS-C total scores though no between group differences existed. For both treatment groups, the K-TEA standard score (SS) improvements were significant for reading comprehension and reading composite while independently, the ADHD group improved in spelling and the ADHD+RD improved in reading decoding. Age equivalence improvements were significant for both groups for reading measures and spelling with no differences between groups. On the WMTB-C, the ADHD group showed significant improvement for the central executive total SS and component scores versus improvements on the phonological loop total SS and visuo-spatial sketchpad component scores for the ADHD+RD group when using a repeated measures analysis. Atomoxetine was well tolerated with no differences between groups and commonly reported adverse events similar to those reported in previous studies.
Discussion: These data suggest that atomoxetine was equally effective in treating ADHD symptoms in patients with ADHD and ADHD+RD. Baseline academic achievement scores in reading for the ADHD+RD group were well below the ADHD group but demonstrated comparable endpoint improvements in nearly all subtests. In neurocognitive function measures of the WMTB-C, marked significant improvement split with scores related to central executive function improved for the ADHD group versus the scores related to the phonological loop and the visuo-spatial sketchpad improved for the ADHD+RD group. The phonological, visual-spatial, and central executive tests assess neurocognitive function in different parts of the brain. The meaning of these differences between groups in relation to comparable changes in improvement in ADHD symptoms is unclear but could suggest that the brain regions related to the therapeutic benefit of atomoxetine in reducing ADHD symptoms may be different between the ADHD+RD and ADHD groups.

30. The Effects of Sex and Pubertal Status on the Potentiated Startle Reflex

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Sponsor: Kathleen R. Merikangas

Background: The startle reflex and its modulation by emotional stimuli is a valuable tool in identifying vulnerability to affective and anxiety disorders (Grillon et al, 2003, Clinical Neurophysiology 114:1557-1579). We have adapted the standard adult startle potentiation of threat of shock for children by potentiating startle with threat of air puffs and darkness (Grillon et al, 1998, Journal of Psychophysiology 12:329-337). The results of our earlier collaborative study of startle in children and adolescents suggested that there may be sex and age specific changes in startle reactivity (Grillon et al, 1999, Int J Psychophysiology, 32:63-73). In order to investigate the influence of pubertal development and startle, we conducted a prospective study of a community cohort of children ages 10-13 across a 9-month period.
Methods: Thirty-six females and 31 males were assessed on 4 separate occasions approximately three months apart. Potentiated-startle and contextual fear paradigms were administered at each session to assess startle response to the anticipation of an aversive stimulus and darkness, a contextual stressor, respectively.
Results: Significant interactions were found between pubertal status and startle condition and between sex and startle condition: females had greater startle potentiation than males across both age and pubertal stage, and pubertal maturation was associated with enhanced potentiation of startle.
Discussion: These findings suggest that the emergence of the sex difference in mood and anxiety disorders may be directly related to puberty, rather than age. Future studies should incorporate the knowledge of the neural circuits involved in startle and its manipulation to examine the extent to which such pathways underlie developmental changes in affective reactivity.

31. Autism-Associated Serotonin Transporter Variants Confer Gain of Function Phenotypes Arising through Distinct Mechanisms

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Background: The human serotonin (5HT) transporter (hSERT) is responsible for 5HT inactivation, both synaptically in the CNS and peripherally. Alterations in SERT activity have been implicated in multiple brain disorders, including major depression, obsessive-compulsive disorder (OCD), and autism. Recently, we (Sutcliffe et al, Am J Hum Genet 2005; Prasad et al, PNAS 2005) identified multiple autism-associated variants in the SERT gene (SLC6A4), including four rare coding variants (Gly56Ala, Ile425Leu, Phe465Leu and Leu550Val). These four coding variants are significantly associated with more severe OC behaviors in the subjects with autism. Other studies (Ozaki et al, Mol Psychiatry, 2003; Delorme et al, Mol Psychiatry, 2005) have identified an Ile425Val coding variant in subjects with complex phenotypes including Asperger's syndrome and OCD. Our functional studies indicated that the most common of these alleles, Gly56Ala, imparts elevated basal SERT activity in transfected cells as well as native lymphocytes and displays elevated basal phosphorylation and insensitivity to regulation through PKG/p38 MAPK-linked pathways.
Methods: Functional effects conferred by coding variants were evaluated in vitro through transfection into HeLa cells and from analysis of natively-expressing lymphocytes. SERT-related measures obtained from cells transfected with wildtype and coding mutants included total and cell surface SERT binding, 5HT uptake, and response to regulation by PKC, PKG and p38 MAPK signaling pathways.

Results: HeLa cells transfected with Ile425Leu, Ile425Val, Phe465Leu, and Leu550Val encoded SERT display elevated basal 5HT uptake, arising from an elevated 5HT transport Vmax. Unlike Gly56Ala which displays elevated transport function without changes in surface density, the latter three variants display elevated surface expression that parallels changes in 5HT uptake, suggesting that functional alterations derive from changes in constitutive transporter trafficking. Additionally, Ile425Leu, Ile425Val, Phe465Leu and Leu550Val respond to PKG, p38 MAPK and PKC stimulation similar to wildtype hSERT. Additional studies that probe the mechanism of PKG/p38 MAPK regulation of hSERT as revealed in studies of the Gly56Ala variant will be presented.
Discussion: These studies indicate that although these coding variants impact hSERT in two distinct ways, they all result in increased 5HT transport activity, suggesting that inappropriately elevated 5HT clearance and diminished extracellular 5HT may be the critical determinant of risk mediated by autism- and OCD-associated SERT alleles. Considering the phenotypes associated with the Ile425Val mutation, these findings further support the premise that autism, OCD, and related disorders share etiological factors.

32. Emotion Regulation Network in Patients with Coronary Artery Disease and Depression: A Structural Magnetic Resonance Imaging Study

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Sponsor: Ellen Frank

Background: Studies suggest relationships between Coronary Artery Disease (CAD) and Major Depressive Disorder (MDD) that may involve abnormalities in emotion regulation. One of the potential markers of emotion regulation, namely, heart rate variability is decreased in CAD+MDD versus non-depressed CAD patients. The aim of this study was to examine structural alterations in emotion regulation circuitry in patients with CAD+MDD compared to matched healthy control subjects (CTRL), CAD and MDD patients. We hypothesized that subjects with CAD+MDD would have more prominent alterations in the grey matter of brain regions that are implicated in emotion regulation, such as the amygdala, insula and orbitofrontal cortex, compared to MDD and healthy control (CTRL) subjects. We also explored differences in grey matter changes between CAD, MDD and CTRL groups.
Methods: We recruited subjects with CAD + MDD (n = 12), CAD (n= 12), MDD (n = 19), and age-matched healthy CTRL subjects (n = 17). We acquired structural magnetic resonance imaging scans using a uniform protocol across the groups on a 1.5T GE whole body scanner. We performed a voxel-wise ANCOVA of normalized smoothed grey matter maps, with age and gender as covariates, using an optimized voxel based morphometry protocol on statistical parametric mapping (SPM5) software (threshold p = 0.001; minimum cluster size = 30 voxels). MNI coordinates were converted to Talairach coordinates to identify the brain regions with significant grey matter changes.
Results: We found decreased grey matter volumes in the bilateral amygdala (T = 3.65), right insula (T = 4.22) and left ventral prefrontal cortex (BA 10; T = 4.36) in the CAD+MDD group relative to the CTRL group (uncorrected p<0.001 for all structures). These differences remained at a trend level following conservative corrections for multiple comparisons across the whole brain, at p =0.058 (False Discovery rate correction). Our exploratory analyses did not find significant differences in grey matter volumes between CAD+MDD and MDD groups, CAD+MDD and CAD groups, CAD and CTRL groups, and MDD and CTRL groups.
Discussion: Our preliminary analysis support our hypothesis that, relative to CTRL subjects, CAD+MDD patients may have impairment in a specific network of brain regions involved in emotion regulation. Our exploratory analysis found no significant differences in grey matter between CAD, MDD and CTRL groups. These findings offer initial clues that CAD+MDD patients may have abnormalities in the emotion regulation network.

33. Brain Structure and Symptom Dimension Relationships in Obsessive Compulsive Disorder (OCD): A Voxel-Based Morphometry (VBM) Study

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Sponsor: Travel Awardee, Young Investigator Award, 2006

Background: While neuroimaging studies have identified functional neural correlates of OCD symptom dimensions, patterns of structural differences associated with symptom dimensions remain to be fully elucidated. Voxel-based morphometry (VBM) assesses differences in gray matter concentration across the whole brain. This study was designed to use VBM to measure relationships between brain structure and symptom dimensions in adults with OCD.
Methods: Adult OCD subjects (N=25) were first compared to healthy controls (N=20). Subject symptom dimension severity scores were then measured and subjects were subdivided into low vs. high score groups. T1-weighted SPGR images (124 1.5mm thick coronal slices) were acquired on a 1.5T G.E.system. VBM analysis was conducted using SPM 5. The images were spatially normalized and then segmented into gray, white and CSF compartments using probabilistic classification. A preset threshold (p<0.001, uncorrected) was employed to identify suprathreshold voxels. Statistical comparisons were performed with the general linear model, implementing small volume random field corrections for a priori regions of interest.
Results: VBM analysis revealed significant gray matter reductions in Brodmann Area 9 (BA 9) associated with checking (low>high; p=0.04, corrected) and washing (low>high; p=0.05, corrected) symptoms and in BA 11 associated with hoarding (low>high; p=0.02, corrected) symptoms. Increased gray matter in caudate (p=0.003, corrected) and putamen (p=0.019, corrected) regions were associated with greater hoarding symptom severity (high>low). There were no significant gray matter increases in any brain regions associated with either washing or checking symptom severity.
Discussion: These findings suggest that, consistent with prior studies, hoarding may represent a functionally and structurally distinct symptom dimension of OCD. The results support recent neuroimaging data by providing converging evidence for specific neural mediators of OCD symptom dimensions.

34. PET Study Suggests Differences in Serotonergic Neurotransmission Between Men and Women

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Sponsor: Goran Sedvall

Background: Epidemiological studies indicate significant differences between women and men in the prevalence of serotonin related disorders like depression, anxiety, or suicidal behavior. Despite this, very few studies focus on differences in the serotonin system between sexes. Of the serotonin receptors, serotonin 1A (5-HT1A) has been implicated in the patophysiology of major depression and anxiety disorders. These disorders can successfully be treated with selective serotonin reuptake inhibitors, psychotropic drugs acting via the serotonin transporter (5-HTT). The aim of this study was to evaluate the

sex related differences in 5-HT1A receptor and 5-HTT binding potential (BP).
Methods: Using positron emission tomography and the radioligands [11C]WAY100635 and [11C]MADAM, the binding potentials for 5-HT1A receptors (14 women and 14 men) and 5-HTT (9 women and 10 men), were assessed in the frontal cortex, anterior cingulate, temporal cortex, insula, hippocampus and dorsal raphe. The regional BP values were calculated applying the simplified reference tissue model. The results were analyzed using the Linear mixed effects model which showed significant differences between women and men in both 5-HT1A and 5-HTT BPs. The p values were Bonferroni corrected.
Results: For the 5-HT1A, with regard to regions, there was an indication of systematically heterogenous means between gender ("gender by region", p=0.061). The beforehand planned comparisons examining the effect of gender on BP, showed a significantly higher 5-HT1A receptor BP in females in the region of hippocampus (mean diff.:1.76 [0.0091;3.50], p=0.046) compared to men. An indication of higher BP in females was found in the cingulate (p=0.054) and insula (p=0.093). For the 5-HTT, no proofs were found of heterogeneous means between women and men regarding the regions (interaction "gender by region", p=0.210). In other words, women had a significantly lower mean 5-HTT BP than men (p=0.003) regardless of region.
Discussion: In conclusion, women and men differ with regard to binding potentials for both 5-HT1A receptors and 5-HTT. The findings suggest that brain serotonergic neurotransmission differ between women and men, which may explain the differences in propensity for serotonin related disorders between sexes.

35. Smoking Regular, but not De-Nicotinized, Cigarettes Saturates α4β2* Nicotinic Acetylcholine Receptors

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Background: In a recent study using the radiotracer 2-[18F]fluoro-3-(2(S)-azetidinyl-methoxy) pyridine (2-FA) and positron emission tomography (PET), our group demonstrated that cigarette smoking, in amounts used by typical daily smokers, saturates α4β2* nicotinic acetylcholine receptors (nAChRs), the most common nAChR subtype in the mammalian brain. Receptor occupancy was dose-dependent, with the ED50 (effective dose needed to occupy 50% of available α4β2* nAChRs) being 0.13 of a cigarette (roughly 0.17 mg nicotine intake). These data predict that if α4β2* nAChR occupancy is attributable to the nicotine delivered by cigarette smoking, then smoking a de-nicotinized cigarette (0.05 mg nicotine) or a matched low nicotine cigarette (0.6 mg nicotine) would result in 23% or 78% receptor occupancy, respectively.
Methods: Five tobacco-dependent cigarette smokers underwent bolus-plus-continuous-infusion 2-FA PET scanning sessions, during which they smoked either a de-nicotinized or a matched low nicotine cigarette (double-blind, randomized order), following two nights of smoking abstinence. An additional nine scanning sessions were performed on tobacco-dependent smokers using the same method, with either no smoking, smoking a regular cigarette (1.2 to 1.4 mg nicotine), or smoking to satiety (2.5 to 3 regular cigarettes) during scanning (n = 3 for each group).
Results: α4β2* nAChR occupancy by smoking was commensurate with the amount of nicotine contained in the cigarette smoked. Levels of α4β2* nAChR occupancy from smoking a de-nicotinized cigarette and a matched low nicotine cigarette were 27% and 77%, respectively (compared with 88% occupancy from smoking a regular cigarette and 95% occupancy from smoking to satiety).
Discussion: Smoking de-nicotinized or low nicotine cigarettes results in levels of α4β2* nAChR occupancy consistent with the nicotine content of these cigarettes, while smoking regular cigarettes results in saturation of α4β2* nAChRs. These findings indicate that other aspects of smoking (e.g., inhalation of non-nicotine components of tobacco smoke or physiological responses to conditioned cues) are not responsible for changes in α4β2* nAChR occupancy with cigarette smoking.

36. A Voxel-Based Morphometry Study of Complex Genetic Interactions in Catechol-O-Methyltransferase (COMT)

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Background: COMT is a catabolic enzyme critical for degrading extracellular dopamine in neocortex. A widely studied functional single nucleotide polymorphism (SNP), Val158Met (rs4680), has been found to impact enzyme activity, prefrontal cortical dopamine and function, and risk for schizophrenia. Controversy exists, however, regarding the contribution of individual alleles and haplotypes, suggesting that additional genetic variability in COMT may be important. The study of potentially interacting SNP loci within a gene requires examination of haplotypes (alleles coinherited, usually due to spatial proximity on the same chromosome), which is technically challenging since they can only be partially resolved using conventional genotyping techniques. We recently developed a method to achieve this goal, applied here to investigate brain structure using voxel-based morphometry (VBM) in a large sample of 132 (M: F 65:67) healthy volunteers.
Methods: We studied a 2-SNP haplotype composed of Val158Met and a putative P2 promoter region SNP (rs2097603) previously found to impact on enzyme activity, possibly by influencing expression (the rarer, 2-allele associated with less expression and activity than the 1-allele). T1-weighted images were acquired on a 1.5T GE scanner and VBM was performed in SPM2 using an optimized VBM protocol with customized apriori templates. An adapted haplotype trend regression model, specified in SPM2, was used to test for the association between absolute gray matter volume and estimated haplotype frequencies, with age, IQ, and sex as covariates. An additional analysis, including total gray matter volume as a confounding covariate, was used to assess relative gray matter volume differences.
Results: Examination of the 2-SNP haplotype revealed a significant effect for variation of Val158Met on the P2 promoter-1 background ($p < 0.06$, $t = 4.78$, whole-brain corrected, $p < 0.011$, $t = 3.73$, corrected in hippocampal ROI, MNI coordinates ((-35, -16, -8) & (-34, -14, -13), respectively). Inspection of absolute and relative volumes showed that haplotype effects, if ordered by assumed impact of genetic variation on enzyme activity, showed a nonlinear relationship with both 1-2 and 2-1 being associated with lower volumes than 1-1 and 2-2 (corresponding to highest enzyme activity for 1-1, then 2-1 (effect of reduced expression at promoter SNP), 1-2 (corresponding to rs4680 met on promoter 1 background) and finally 2-2). A similar finding was observed in the right hippocampus. Results from the relative gray matter analysis were similar in effect, though slightly less significant in the hippocampus ($p < 0.06$, $t = 4.91$, whole-brain corrected, $p < 0.025$, $t = 3.64$, corrected in ROI (-32, -17, -10)). In addition to the hippocampus, exploratory analyses showed effects on cortical gray matter volume in the right medial prefrontal cortex, the left superior temporal gyrus, and the right occipital lobe.
Discussion: Our data reveal a significant effect of genetic variation in COMT on hippocampal volumes, suggesting that extracellular dopamine may have effects on brain structure. This is neurobiologically plausible since the hippocampus expresses COMT, dopamine impacts brain development and is implicated in ongoing local neural plasticity. Our findings replicate a previous observation (in functional data) of a "u-shaped" nonlinear contribution of interacting loci on the COMT gene. This nonlinear interaction may contribute to previous conflicting findings of volumetric differences when only

Val158Met was examined. Our results support the concept of an optimum range of extracellular dopamine concentration that leads to volume abnormalities, either when not reached (1-1) or markedly exceeded (2-2).

37. Functional Neuroimaging Studies of the Human Insula

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Background: The human insula is implicated in an array of cognitive–emotional–somatic processes. These include gustatory sensory, motor, somatosensory processing; representation of peripheral autonomic states, processing negative emotions, recall/imagery of stressful states, and cognitive operations with and without emotion components. Rich reciprocal connections link the insula with the prefrontal cortex (OFC, ACC), somatosensory cortex, parietal cortex, temporal pole and the amygdala, making it well positioned to contribute to the integration of cognitive, emotional and somatic information in the service of generating a coordinated response.
Methods: We selectively reviewed data related to human insular function acquired in a series of fMRI and PET studies conducted by our group over the past 8 years in healthy subjects and subjects with PTSD utilizing emotional probes (emotional faces, IAPS pictures, and stressful/traumatic autobiographical narratives). In some of the studies participants also completed self-administered personality questionnaires including NEO-PI (from which we derived Neuroticism and Extraversion scores) and/or TPQ (Harm Avoidance), and TAS-26 (Alexithymia). In a PET study of emotional processing in PTSD, we also sampled venous blood every 5 min and measured ACTH and cortisol.
Results: Left insula [(-39, 18, -9), z=3.56] was activated by emotional faces but not by IAPS picture in an fMRI study. Insula activation was seen in response to happy [(-30, 6, 9), z=2.62]; sad [(51, 12, -2), z=2.6], [(-42,21, -21), z=3.53]; angry [(-39, 18, -9), z=3.86]; and fearful [(-36, 15, 6), z=3.61] faces (versus neutral faces), but not to IAPS pictures. In another study comparing social vs. nonsocial emotional film stimuli, insula activation was seen in non-social positive (appetite) [(-36,-24,0),z=3.00], (33, -15, 6), z=3.99] and non-social negative (disgust) [(33,-15,15), z=3.44], but not in social stimuli (comedy or sad). In the PET study, bilateral insula was activated by traumatic/stressful scripts (neutral scripts contrast) in all subjects (healthy non-combat controls [(34,4,12), z=4.47], combat-exposed controls [(40, -12, -6), z=2.59], and combat PTSD [(38,2,2), z=3.27] and ACTH responses correlated with rCBF in right anterior insula [(42, 28, 4), z=4.13]; [(34, 28, -16), z=3.37] and posterior insula [(48,-40,16), z=3.31]. Harm avoidance also correlated with rCBF responses to trauma script in right anterior insula/BA13 [(44, 24, 12), z=4.01 in combat-exposed subjects (n=31). An analysis of combined data from three independent studies (38 healthy subjects) involving passive viewing/appraisal of emotional stimuli found that extraversion scores inversely correlated with BOLD activation in bilateral anterior insula [left: (-33,18,6), Z=2.67; right: (33,27,-9) Z=2.91] in response to negative relative to neutral stimuli. Alexithymia scores directly correlated with posterior insula activation in response to positive: [(-42,-12,12), Z=3.96]and negative [(-42,-12,-6), Z=3.30] films. Finally, a metanalysis of 55 neuroimaging studies conducted by our group also found that induction by emotional recall/imagery and emotional tasks with cognitive demand recruited the insula.
Discussion: Most of our knowledge of the role of the insula arises from animal research, although human neuroimaging research is beginning to accumulate and clarify the putative roles of the insula in complex cognitive-emotional-somatic interactions. The evidence seems to support the role of the insula in integrative cognitive, emotional and somatic processing. The emergence of insular findings in neuroimaging studies of stress and anxiety disorders such as PTSD and phobias warrants a more systematic approach to studying the human insula in health and disease.

38. Pilot fMRI Study of Prosody in Schizotypal Personality Disorder: Sarcasm Is Difficult to Process

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Sponsor: Robert W. McCarley

Background: Schizotypal personality disorder (SPD) is characterized by social anxiety, impoverished friendships, and odd speech/language. One aspect of language critical to social reciprocity is prosody, that is, changes in vocal inflection needed to convey meaning. The superior temporal sulcus (STS) primarily on the right, is considered important for the processing of prosody.
Methods: In this pilot study we explored fMRI activation patterns for SPD (N=3) and control (N=3) subjects as they heard 30 semantically neutral sentences read in a happy, sad, sarcastic, or neutral tone of voice (15 sentences/emotion/run =60 sentences/run for 2 runs, order counterbalanced). Acquisition parameters: TR=2, 24 5mm slices AC-PC, 285 acquisitions, run time=9min 31s. Contrast images for neutral, all emotions (happy, sad, and sarcastic), and each emotion separately, were created for the two diagnostic groups. The extent of activation on combined left and right were compared at threshold $p<0.001$, using a fixed-effects model. On structural MRI images 1.5mm thick, the STS was manually drawn and compared between groups using ANOVA (SPD N=19 and control N=14).
Results: One-sample t tests for control subjects revealed the expected right greater than left extent of activation in the region of the STS for each contrast. SPD subjects did not show that normal asymmetry, instead, had a left greater than right extent of activation for each contrast. In no region did control subjects activate more for emotional vs. neutral sentences, whereas SPD subjects also recruited the left prefrontal cortex for emotional vs. neutral sentences. In the region of the combined left and right STS, control subjects had a 6.9% greater extent of activation while processing emotional sentences compared with neutral sentences, whereas SPD subjects had a 36% increase. In SPD subjects, this was mainly due to the effect of the sarcastic condition (41% increase of bilateral extent of activation compared with the neutral condition). There was no difference between groups in terms of reaction time or number of correct responses, suggesting that both groups were able to perform the task. There was also no difference between groups on STS volumes (interrater intraclass reliability >0.9).
Discussion: SPD subjects had abnormal asymmetry and inefficient processing of prosodic sentences. This is unlikely due to differences in volumes of regions considered key for processing of prosody. Moreover, more subtle emotions, such as sarcasm, enlist the largest extent of activation for SPD subjects. If these results hold as we increase our subject N, then they may point to an area for potential social remediation.

39. COMT val158met Polymorphism and Pain Processing in Borderline Personality Disorder

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Sponsor: James Douglas Bremner

Background: A functional polymorphism (val158met) of the gene coding for Catechol-O-methyltransferase (COMT) has been demonstrated to be related to processing of emotional stimuli. Also, this polymorphism is associated with pain regulation in healthy subjects. Borderline Personality Disorder (BPD) is characterized by emotional dysregulation and reduced pain sensitivity. Therefore, we investigated associations between COMT genotypes and pain processing in patients with BPD.

Methods: 24 female patients fulfilling DSM-IV criteria for BPD were included in this study. The number of val158 alleles was correlated with the fMRI BOLD response during painful thermal stimulation. The analysis of genotype effects was restricted to brain areas with activation by pain.
Results: Painful heat stimuli activated regions in the insula, dorsolateral and ventrolateral prefrontal cortex, parietal lobe and midcingulate. In insula, dorsolateral prefrontal cortex and midcingulate the number of val158 alleles was positively correlated with the BOLD response.
Discussion: Genetic variation in the COMT gene contributes to inter-individual differences in neural pain processing in patients with borderline personality disorder.

40. Abnormal Fronto-Temporal Cortical Activation During fMRI Attention and Working Memory Tasks in Prodromal and Early Illness Patients with Schizophrenia

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Background: Functional magnetic resonance imaging (fMRI) studies of schizophrenia have revealed dysfunction of fronto-temporal, fronto-parietal, and thalamo-cortical circuitry subserving higher order cognitive functions such as attention and working memory. Deficits in these and other cognitive functions contribute to poor functional outcomes in schizophrenia and are minimally responsive to antipsychotic medications. Whether these cognitive functions, or the neural circuitry subserving them, are compromised prior to psychosis onset has not been established. Inasmuch as underlying neural circuitry dysfunctions may precede overt cognitive performance deficits, their elucidation with fMRI may incrementally increase our ability to predict which clinically identified patients with prodromal symptoms are at greatest risk for conversion to psychosis, a critical step for development of targeted preventive interventions.
Methods: Ultra-high risk (UHR) patients meeting the Yale PRIME Clinic's Criteria of Prodromal Syndromes, as well as early illness (EI) schizophrenia patients and healthy controls, underwent fMRI during two tasks: 1) an auditory oddball task in which attention is automatically oriented (novel sounds) or deliberately allocated (target tones) toward deviant stimuli, and 2) a Sternberg Item Recognition Paradigm (SIRP) examining dorsolateral prefrontal cortex (DLPFC) activations across varying working memory loads.
Results: For the oddball task, EI patients had reduced activations to novel and target stimuli in lateral and medial temporal lobes and parietal regions normally associated with deviance detection. Other hypoactive regions were DLPFC, limbic areas, and thalamus. Generally, UHR patients showed similar but less pronounced activation deficits, indicating that dysfunction of the neural circuitry subserving deviance detection may predate, and reflect risk for, psychosis. The SIRP working memory task showed EI patients to be "inefficient" at lower memory loads, over-activating DLPFC, but "deficient" at higher loads, under-activating DLPFC. UHR patients tended to show a similar pattern, albeit less pronounced.
Discussion: These preliminary data suggest that fronto-temporal cortical circuitry subserving attention and working memory are compromised in individuals exhibiting prodromal symptoms indicative of risk for subsequent psychosis. The pattern of compromise is similar to that exhibited by patients early in the course of schizophrenia, although the abnormalities are less pronounced. These abnormalities in prodromal patients may reflect impending psychosis as part of the pathophysiological progression to schizophrenia, or they may reflect the genetic vulnerability for schizophrenia; their potential to predict which prodromal patients subsequently convert to psychosis is currently being evaluated.

41. Changes in Brain Metabolites in Frequently Relapsing Bipolar Patients Treated with Long-Acting Risperidone

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Background: Studies of bipolar patients using magnetic resonance spectroscopy (MRS) have shown Glx (glutamate + glutamine + gamma-aminobutyric acid) is increased in gray matter and that treatment alters Glx and myo-inositol (mI) levels. The application of high field strength MRS techniques permit the quantification of individual components comprising the Glx peak . Atypical antipsychotic medications have been shown to be effective in the treatment of bipolar disorder, however, their neurochemical effects in this disorder are not well characterized.
Methods: Spectra were obtained for 16 subjects with frequently relapsing bipolar disorder enrolled in an ongoing clinical trial of long-acting risperidone (LAR) augmentation. All subjects continued with their treatment as usual (TAU) and were scanned before and after 16 weeks of augmentation with LAR. Thirteen non-psychiatric control subjects also completed the MRS protocol. All imaging data were acquired on a Varian 4 Tesla scanner using 2D magnetic resonance spectroscopic imaging at either U.C. or McLean Hospital.
Results: Bipolar subjects had lower baseline levels of whole brain white matter glutamine ($p<.01$) and white matter myo-inositol compared to controls ($p<0.03$). Following 16 weeks of LAR treatment, bipolar patients showed significant improvement as measured by the Young Mania Rating Scale (YMRS) ($p<0.001$) and changes in whole brain white matter glutamine concentration were significantly increased ($p=.039$). Grey matter myo-inositol levels in bipolar patients also showed a non-significant increase following LAR treatment.
Discussion: Changes in glutamine and myo-inositol concentration in bipolar patients are consistent with previous investigations suggesting a bioenergetic dysfunction in this disorder. Bipolar patients treated with long acting injectable risperidone demonstrated increased glutamine, which may reflect increased cellular metabolism and improved glutamatergic transmission. Our findings suggest that long acting injectable risperidone impacts neurochemical measures which are associated with symptom improvement. Supported by Janssen, L.P.

42. Attenuation of the Effects of Corticosteroids on the Human Hippocampus with Lamotrigine

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Background: An extensive animal literature suggests that stress or corticosteroid exposure is associated with changes in memory and hippocampal structure, and that agents that modulate glutamate prevent and reverse the effects of corticosteroids on the hippocampus. Examining the effects of corticosteroids on the human hippocampus is challenging since humans cannot ethically be given corticosteroids for extended periods. Our group uses patients receiving prescription corticosteroids for the treatment of medical illnesses to explore the effects of these hormones on the human brain. We previously reported poorer performance on a declarative memory task and smaller hippocampal volumes in patients receiving chronic prednisone therapy than in controls with similar medical histories not receiving prednisone. We also reported significant improvement in declarative memory in prednisone-treated patients given open-label lamotrigine, an anti-seizure medication that inhibits glutamate release. In this report we examine the impact of placebo-controlled treatment with

lamotrigine on declarative memory, hippocampal volume and task-related hippocampal activation.
Methods: Twenty-eight outpatient volunteers taking long-term oral prednisone therapy for medical conditions were randomized to lamotrigine (maximum dose of 400 mg/day) or placebo for 24 weeks. At baseline and week 24 declarative memory was assessed with the Rey Auditory Verbal Learning Test (RAVLT) using alternative versions to minimize learning effects. Hippocampal and total brain volumes were manually traced by blinded raters using BRAINS 2 software. Hippocampal function was assessed with BOLD brain activation in response to a visual scene memory task using fMRI. The fMRI data were analyzed using AFNI software.
Results: Data at the week 24 assessment were available on 18 participants. Participants were 13 men and 5 women, mean age 45.9 ± 12.8 years, current prednisone dose of 14.4 ± 10.4 mg/day with duration of prednisone therapy of 62.0 ± 75.7 months. RAVLT total words recalled data were available on 17 participants and significantly ($p < 0.05$) increased in the lamotrigine group (T score from 37.4 ± 14.2 to 46.9 ± 16.0) as compared to the placebo group (38.4 ± 12.6 to 40.1 ± 11.0). Neuroimaging data were available on 12 participants. Consistent with a reduction in glutamate release, a voxel cluster in the right hippocampal/parahippocampal gyrus region showed a significant baseline to week 24 decrease in BOLD activation during a visual scene memory task in the lamotrigine group as compared with the placebo group as reflected by a Group * Time interaction, $F(1, 10) \geq 6.94$ ($p \leq$ 01 individual voxel threshold; $p \leq 0.05$ overall) reflecting a selective drug effect in the right parahippocampal/hippocampal region, mostly associated with decreased activation in the drug treatment group relative to the placebo group. The lamotrigine group had a mean decrease in total brain volume adjusted right and left combined hippocampal volume of 0.1%, while the placebo group had a mean decrease of 0.8% ($p = NS$).
Discussion: Corticosteroid-treated patients given lamotrigine showed improvement in declarative memory and a decrease in parahippocampal/hippocampal region activation as compared to placebo. RAVLT scores increased from the mildly impaired to the average range in the lamotrigine group while showing little change in the placebo group. Thus, declarative memory changes appear to be clinically significant. Consistent with the data in animal models, these results suggest that agents that modulate glutamate may protect the human hippocampus from the toxic effects of long-term corticosteroid exposure.

43. Escitalopram Attenuates BOLD Activation in Amygdala and Insula During Processing of Emotional Stimuli

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Sponsor: Murray B. Stein

Background: The amygdala and insular cortex are important for the assessment of emotionally salient stimuli. Previous brain imaging studies have shown that individuals with anxiety and mood disorders present increased activation of these limbic structures during emotional processing. Moreover, we have shown in healthy volunteers that an anxiolytic agent, lorazepam, dose-dependently attenuates activation of limbic structures (Paulus et al., 2005; Arce et al., in press). Taken together, these findings provide evidence that pharmaco-functional Magnetic Resonance Imaging (fMRI) may be useful as a biomarker for mood and anxiety disorders. An important next step is to show that commonly used antidepressants such as selective serotonin reuptake inhibitor (SSRI) also affect these neural systems. Therefore, the current study investigated whether administration of escitalopram alters the activation of limbic structures.
Methods: We hypothesized that subchronic SSRI treatment in healthy individuals attenuates the activation of the amygdala and insula during emotional face processing. Thirteen healthy volunteers participated in a double-blind, placebo-controlled, cross-over, randomized study. After twenty-one days of treatment with either escitalopram or placebo, participants underwent fMRI. During fMRI, all subjects completed an emotion face assessment task, which has been shown to elicit amygdala and insula activation.
Results: The main result of this study is a urine-level dependent attenuation of bilateral amygdala and insular cortex. This finding is consistent with the notion that therapeutic agents that reduce anxiety and/or depression modulate activation of limbic structures during emotion processing. Moreover, this attenuation was modulated by individual metabolization rate of the SSRI.
Discussion: The current investigation (1) provides further support for the use of blood oxygenation level-dependent (BOLD) fMRI with pharmacological probes to help identify the specific therapeutic effect of SSRIs in anxiety and mood disorders and (2) highlights the importance of the pharmacokinetics of antidepressants in modulating limbic processing of emotional stimuli.

44. Reduced Ventral Striatal D2/D3 Receptor Binding in Depressed Reproductive-Aged Women: A [^{11}C]Raclopride Positron Emission Tomography Study

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Sponsor: Past Travel Awardee,Young Investigator Memorial, 2005

Background: Dopamine (DA) function in the ventral striatum is associated with reward and pleasure-related behaviors. Because a core symptom of major depressive disorder (MDD) is anhedonia, disrupted ventral striatal DA function has been proposed as a mechanism of MDD. Several in vivo imaging reports of increased DA D2/D3 receptor binding in MDD suggested postsynaptic receptor upregulation in response to low extracellular DA concentrations. We tested the hypothesis of increased striatal D2/D3 receptor binding in MDD versus controls, measured with [^{11}C]raclopride-positron emission tomography (PET).
Methods: Postpartum and non-postpartum women were enrolled. Nine unipolar depressed (MDD) and 27 healthy control (HC) women underwent a 60-min dynamic PET scan using a Siemens/CTI HR+ upon i.v. administration of [^{11}C]raclopride. Regions of interest (ROIs) included anteroventral striatum (AVS), dorsal caudate (DCA), dorsal (DPU) and ventral putamen (VPU), with cerebellum (CER) as the reference region. Binding potential (BP) was determined using a simplified reference tissue method (SRTM). Statistical inference was conducted with paired t-tests, with significance threshold set at $\alpha \leq 0.0125$ (to control for multiple comparisons). Linear regression was used post-hoc to evaluate significance of the depression effect while covarying for age.
Results: Depressed subjects were either antidepressant naïve (n=6) or free from antidepressants > 16 weeks. Subject groups did not differ on the basis of age (HC: 27.8 ± 7.5, MDD: 30.4 ± 7.0; p=0.37), proportion of African American subjects, smokers, postpartum women (HC: 30%, MDD: 56%; p=0.16), breastfeeders, duration postpartum (weeks; HC: 11.2 ± 2.5, MDD: 12.6 ± 1.8; p=0.31), day of menstrual cycle (HC: 6.9 ± 4.3, MDD: 5.0 ± 4.0; p=0.37), or estradiol (pg/ml; HC: 61.1 ± 89.8, MDD: 66.3 ± 12.1; p=0.91) or progesterone concentrations (nmol/L; HC: 1.1 ± 0.86, MDD: 1.3 ± 2.1; p=0.69). Subject groups did not differ on the basis of injected [^{11}C]raclopride dose, CER DV (HC: 0.42 ± 0.09, MDD: 0.42 ± 0.04; p=0.96), or RI (radiotracer delivery to ROI relative to CER). D2 receptor BP was significantly lower in the MDD relative to HC group in AVS (28% reduction; HC: 2.09 ± 0.37, MDD: 1.50 ± 0.47; p<0.0001) and VPU (18% reduction; HC: 2.87 ± 0.38, MDD: 2.34 ± 0.54; p=0.003). There were no significant group differences in DCA (8% reduction; HC: 2.25 ±

0.28, MDD: 2.07 ± 0.37; p=0.14) or DPU (5% reduction; HC: 2.97 ± 0.41, MDD: 2.82 ± 0.43; p=0.38). Correction for partial volume effects did not change these observations. D2 receptor BP was best predicted by age and depression in AVS [F(2,33)=12.52 ; p<.0001 ; r2=0.43] and VPU [F(2,33)=14.50 ; p<.00001 ; r2=0.47]. For every decade of life, ventral striatal D2 receptor BP was reduced by 0.2-0.3. Women with depression had 0.44-0.53 reductions in ventral striatal D2 receptor BP compared to controls across age.
Discussion: Ventral striatal D2 receptor BP was reduced 18-28% in this sample of reproductive-aged women with scan acquisition at times of low circulating ovarian hormones. These data suggest that reduced ventral striatal D2 receptor density or affinity may be a mechanism or an effect of depression in women. This is consistent with animal models of depression wherein chronic stress induces reductions of D2 receptor mRNA. Our findings contrast with reports of increased or equivalent striatal D2 receptor BP in depressed versus control subjects and may be accounted for by the ability to resolve the ventral from dorsal striatum, study of a homogenous sample, control for circulating ovarian hormones, or unique DA pathophysiology in depressed women. Support: NARSAD, MH64561.

45. Cognitive Inhibition Suppresses Brain Metabolic Response to Food Presemtation

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Sponsor: Nora D. Volkow

Background: The ability to control and regulate impulses, emotions, and desires is one of the core features of the self. Impairment of impulse control has been linked to a broad spectrum of psychopathologies and problems including obesity. The neurobiological mechanism underlying the modulation of self-control is poorly understood. Here we assess the brain circuits involved attempted inhibition during food stimulation using PET and FDG.
Methods: Seven female and 9 male healthy subjects (31.9 ±7.7 yrs of age) with body mass index of 24.4±2.7 (range 21-30) were evaluated with PET and FDG. Brain metabolism was evaluated in food deprived (17-19 hours) subjects during baseline (no stimulation: NS) and food presentation (FP) with and without cognitive inhibition (CI) conditions in 3 separate days. The absolute metabolic images were analyzed using Statistical Parametric Mapping and ROI methods. Self-reports for feelings of "hunger" and "desire for food" were measured on a scale of 1-10.
Results: The subjects had significantly lower scores in "desire for food" during CI (6.4±2.6) than without CI (9.6±0.9, $p < 0.0002$). Whole brain metabolism was significantly higher for FP than for NS (35.6±4.7 µmol/100g/min) but whole brain metabolism did not differ for FP with CI (40±6.6) versus FP without CI (43.4±10 µmol/100g/min). However, there were significant differences in the regional pattern of activation for FP without CI versus FP with CI. Whereas FP without CI led to significant activation ($p < 0.005$) in somatosensory cortex, visual cortex, cerebellum, thalamus, rectal gyrus, left anterior cingulate, left orbitofrontal cortex (OFC) and left insula. FP with CI activated only somatosensory cortex, visual cortex and cerebellum. Right OFC activitation during FP without CI, was associated with significant increases in self-reports of hunger ($r = 0.54$; $p < 0.03$).
Discussion: Suppression of activation in the thalamus, rectal gyrus, anterior cingulate, OFC and insula during FP with CI, which are regions involved in the regulation of satiety and motivation to eat suggests that this is the mechanism by which cognitive inhibition decreases the desire for food. The significant association between OFC activation and hunger corroborates the importance of this brain region in processing motivational aspects of food consumption. Supported by DOE (OBER), NIAAA (Y1AA3009-04) and NIH/GCRC (5-MO1-RR-10710).

46. The Temporal and Extrastriatal D_2/D_3 Receptor Binding Profile of Aripiprazole in Patients with Schizophrenia

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Background: It is now widely accepted that the antipsychotic effects of dopamine receptor antagonists occur within a "therapeutic window" between 60 and 80% D_2 receptor occupancy. The incidence of extrapyramidal side effects increases above the 80% threshold (Farde et al., Arch Gen Psychiatry 1992, 49: 538-544). Although clozapine and quetiapine seem to be exceptions, this rule does also apply for most of the "atypical" antipsychotics. However, our [^{11}C]raclopride PET study in normal volunteers to determine the optimal dose of aripiprazole for clinical trials in schizophrenia demonstrated that these rules apply to antagonists only. Here we showed that aripiprazole occupies more than 90% of striatal D_2 receptors at clinically effective doses without extrapyramidal side-effects (Yokoi et al., Neuropsychopharmacology 2002, 27: 248-259; Gründer et al., Arch Gen Psychiatry 2003, 60: 974-977). In order to further characterize aripiprazole's extrastriatal and temporal binding characteristics, we performed PET studies with [^{18}F]fallypride ([^{18}F]FP) in patients with schizophrenia at varying time points after the last drug administration.
Methods: D_2-like dopamine receptors were quantified with positron emission tomography and [^{18}F]FP in 12 patients suffering from schizophrenia (DSM-IV). The PET scans were performed at varying time points after the last drug administration (range 5-78 h). Time activity curves were generated after normalization using a template for cerebellum, caudate nucleus, putamen, temporal and frontal cortices, thalamus, amygdala, pituitary, colliculi, and substantia nigra. Binding potentials were calculated by means of the simplified reference tissue model. Receptor occupancy was calculated as percent reduction in binding potential, with unblocked values taken from a sample of 12 age-matched normal volunteers. Aripiprazole plasma concentrations were determined immediately before injection of the radiotracer. Plasma concentrations and percent binding data were fit to a simple one-site ligand binding model by nonlinear regression.
Results: Analysis of the data of six of the patients revealed very high mean D_2/D_3 receptor occupancies in all brain regions (mean ± standard deviation, putamen 80 ± 11%, range 60-92%; caudate 83 ± 9%, range 66-93%; thalamus 80 ± 9%, range 68-90%; superior temporal cortex 79 ± 9%, range 70-90%), with slightly higher values in extrastriatal regions at very low plasma concentrations only. D_2/D_3 receptor occupancy was still in the range between 71 and 83% in a patient who had received his last dose 78 h prior to the PET scan. Aripiprazole plasma concentrations ranged from 27 ng/ml to 484 ng/ml. Nonlinear regression analysis revealed E_{max} (maximum attainable receptor occupancy) values close to saturation in all brain regions. EC_{50} (plasma concentration predicted to provide 50% of the maximum attainable occupancy) values ranged from 4 ng/ml in the superior temporal cortex to 14 ng/ml in the putamen.
Discussion: Our preliminary analyses suggest that aripiprazole due to its high affinity to D_2/D_3 receptors and its very long elimination half-life of about 72 hours at clinically used doses occupies very high amounts of its target receptor homogenously throughout the brain and that dissociation from those receptors is very slow. It can be calculated from our results that in patients with plasma concentrations above approximately 400 ng/ml D_2/D_3 receptors are still almost saturated for nearly one week after the last dose.

47. Alpha7 Nicotinic Cholinergic Receptor (CHRNA7) Polymorphisms Discriminate Figural Memory Abilities in Healthy Adults and Influence Related Structural and Functional MRI Patterns

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Background: The role of the alpha7 nicotinic cholinergic receptor (CHRNA7) has been studied mainly in relation to sensory gating phenomena and attention, particularly in schizophrenia, but its role in normal memory function is less explored.
Methods: We studied the relationship between memory, fMRI brain activation, MRI gray matter, and single nucleotide polymorphism (SNP) genotypes at the CHRNA7 locus. Thirty healthy adults from a community study of aging were genotyped at a cytosine-to-thymine SNP (rs868437) and a adenine-to-guanine SNP(rs2337506) at the CHRNA7 locus; they underwent cognitive testing, a functional (fMRI) figural memory paradigm (FIGMEM) where stimuli were abstract shapes designed to have low verbal encodability and a structural MRI scan.
Results: SNP rs868437 differentiated scores on in-scanner FIGMEM testing. SNP rs2337506 also showed a trend to differentiate FIGMEM scores, which varied with rs868437 genotype. CHRNA7 genotype effect was specific to FIGMEM in that it did not discriminate out-of-scanner IQ scores or memory scores from the Mini Mental Status Examination, or the Wechsler Memory Scale-Revised and had only a weak effect on attentional measures. Subjects who scored poorly on FIGMEM had greater regional brain BOLD activation than did the high-scoring group during encoding and recognition. Principal Component Analysis followed by hierarchical regression found that genotype and fMRI activation during the FIGMEM encoding period accounted for as much as 50% of the variance in the prediction of FIGMEM recognition scores. Both posterior (fusiform gyrus, inferior occipital gyrus, and the precuneus) and anterior (medial frontal gyrus, caudate body, and paracentral lobule) brain regional activations were associated with FIGMEM scores. We also used voxel-based morphometry (VBM) to compare the CHRNA7 rs868437 SNP variants in terms of gray matter (GM) distribution in an extended normal population (N=66) including the 30 subjects above. Subjects with he better performing 't' allele had more GM in visual association and hippocampal regions. In the original N=30 sample we found a correlation between each subject's individual GM values in visual association cortex and their FIGMEM performance. Thus, the same CHRNA7 SNP that was associated with better FIGMEM task performance and with less fMRI BOLD activation in this region was also associated with more regional gray matter in these subjects.
Discussion: Overall, these findings suggest that CHRNA7 receptors have a significant role in fostering nonverbal memory consolidation in cognitively normal adults, influencing both brain structure and brain function in task-associated brain regions.

48. Altered Response to Sweet Stimuli After Recovery From Restricting Type Anorexia Nervosa

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Individuals with Anorexia Nervosa (AN) appear to have an altered hedonic or even aversive appreciation of normally pleasurable foods. Several lines of evidence suggest AN women have aberrant dopamine (DA) function. Palatable foods are known to be associated with DA release, thus suggesting a mechanism that may contribute to pathological feeding in AN. This study seeks to understand the neurocircuitry involved in salient sweet stimuli processing in AN, by asking whether there is an aberrant response to sweet taste in general, or more specifically to caloric sugar compared to unmetabolized artificial sweetener.
Methods: In order to avoid the confounding effects of malnutrition, we compared 6 women who were recovered from AN to 13 age matched control women (CW). All subjects underwent blind taste tests for sucrose (concentration range 0-32%) and an artificial sweetener (splenda) to assess sweetness and pleasantness response. For the fMRI study, the artificial sweetener concentration was blindly matched to 10% sucrose solution so that subjects could not distinguish the two tastes. Subjects underwent fMRI scanning and received the two matched sweet tastes in pseudorandom manner in order to test for error prediction signals of DA innervated brain regions. Condition main effects (p 0.001) and comparisons for taste condition and group (p 0.05) were assessed (AFNI software, multiple comparisons corrected with 8 contiguous voxel threshold and sphericity correction).
Results: In this preliminary analysis, AN subjects needed 30% greater amounts of artificial sweetener in order to match a 10% sucrose intensity compared to CW (Mann-Whitney U=19.5, p=0.02). Within group main effects showed that in CW and AN, both sucrose and artificial sweetener activated sensorimotor cortex, insula, thalamus and substantia nigra. In CW, greater activation in the orbitofrontal cortex was found for sucrose versus artificial sweetener. In this small sample of AN, no significant difference was found for sucrose versus artificial sweetener. Irrespective of condition (sucrose or artificial sweetener), AN subjects had greater activation in the ventral and middle striatum, as well as insula and midbrain compared to CW.
Discussion: These preliminary results indicate that AN have an altered perception of sweet taste. Behaviorally, they required more artificial sweetener to match a 10% sucrose concentration. Second, AN subjects had an exaggerated hemodynamic response to either artificial sweetener or sucrose in the ventral striatum and midbrain structures, which suggests an altered response of DA pathways. The increased responsiveness in AN subjects to the nutritious and non-nutritious salient stimulus could indicate a generalized increased sensitivity to saliency.

49. Prefrontal Dopaminergic Modulation of Updating but not Retrieval in Working Memory – A fMRI Study on the Catechol-O-Methyltransferase Val158Met Polymorphism

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Background: Encoding, retrieving, manipulating and temporally integrating information in working memory are important facets of computations necessary to optimize a range of goal-directed behaviors. A wealth of data implicates dopamine in the working memory in general, although less is known about the specific functional anatomy of sub-processes in relation to dopaminergic modulation in vivo. Here, we hypothesized that if individuals with the COMT Val-allele have relatively reduced cortical synaptic dopamine (Chen et al Am J Hum Genet 2004), then working memory sub-processes associated with increased dopaminergic modulation might result in relatively greater task-specific activation in these individuals.
Methods: We explored these working memory stages in event-related fMRI using a numerical working memory task in 22 healthy subjects, with relative cortical synaptic dopamine indexed by the COMT Val158Met polymorphism. Statistical thresholds were set at $p<0.05$ corrected for the search volume in the prefrontal regions-of-interest.
Results: At the baseline numerical size judgment task, Val-allele load was associated with increased ventrolateral prefrontal cortical activation. During working memory encoding, but not at retrieval, increased Val-associated activation was observed in the

dorsolateral prefrontal cortex, although both of these task phases activated the dorsolateral prefrontal cortex. Manipulating numerical information, and temporally integrating information in working memory were incrementally more strongly associated with dopaminergic modulation within the dorsolateral prefrontal cortex.
Discussion: These findings are consistent with suggestions that dopamine is critical in signaling salient change or updating in information processing, such as in encoding and manipulating information, and less so retrieving already stabilized representations. Moreover, complex working memory operations were associated with more pronounced dopaminergic modulation that extends to the dorsal prefrontal regions. Presumably, increased task-related activation with Val allele-load could reflect the greater physiologic dopamine response to decreased synaptic dopamine in these individuals.

50. Neural Correlates of Emotion Dysregulation in Borderline Personality Disorder
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Sponsor: Larry J. Siever

Background: Affective instability, a hallmark feature of borderline personality disorder (BPD), is associated with many of its most disabling symptoms such as suicidality, inappropriate anger, stormy interpersonal relationships, and identity disturbances, yet the mechanism of emotional dysregulation in BPD is poorly understood. One possible explanation is that BPD patients can not modulate their emotional reactions as effectively as healthy individuals. The present study employs fMRI to compare regional activation in BPD patients and healthy volunteers as they employ cognitive reappraisal strategies to down-regulate their emotional responses to negative stimuli. We hypothesized that BPD patients would be less able to activate brain regions involved in emotional control than healthy volunteers as they attempt to down regulate their response to negative emotional stimuli.
Methods: BOLD fMRI images were acquired at 3.0 T while 8 BPD patients and 8 healthy volunteers (HC's) followed instructions to either maintain or suppress their emotional reaction while viewing emotionally negative images, depicting interpersonal senarios, selected from the International Affective Pictures System (IAPS). During the scan subjects provided real-time ratings of their reactions to each picture after carrying out the suppress or maintain instruction. Activation data were analyzed with SPM2, comparing the activations in the suppress minus maintain conditions between groups.
Results: Behavioral data confirmed that both groups rated pictures as less negative during suppress trials than maintain trials and that the difference between suppress and maintain trials was greater for HC's. The HC's showed greater activation in the suppress relative to maintain condition, compared to the BPD's, in the anterior cingulate cortex (ACC; MNI Coords: 0,31,28; p_{uncorr} <.01, cluster size >100), the pregenual anterior cingulate cortex (MNI coords: 6,49,-2; p_{uncorr} <.05, cluster size>50), and the intraparietal sulci (IPS) bilaterally (MNI coords:28,-56,38; p_{uncorr} =.05, cluster size>50) and a trend for increased activation in the tempero-parietal junction (MNI coords: -52,-46,30). The BPDs demonstrated greater activation in the striatum bilaterally than the HC's (p_{uncorr} <.01, cluster size>100).
Discussion: These pilot findings suggest that BPD patients activate brain regions implicated in control of emotion (dorsal and pregenual ACC) and in attentional disengagement (IPS) to a lesser degree than healthy volunteers when trying to suppress their emotional reactions to negative stimuli.

51. The Effect of Aripiprazole on Cue-Induced Brain Activation in Alcoholics
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Sponsor: Raymond F Anton

Background: Functional imaging techniques have been increasingly used to understand the neurobiology of addictive disorders. Using fMRI, our group found significant brain activation in the anterior cingulate, insula, nucleus accumbens, and ventral tegmental area in non-treatment seeking alcoholics, but not social drinkers, after a sip of alcohol and during the viewing of alcohol pictures (Myrick et. al 2004). The rewarding aspects of alcohol consumption are thought to be medicated by increased dopamine in the nucleus accumbens.The goal of the study was to utilize this imaging paradigm to explore the effect of aripiprazole, a dopamine stabilizer medication, on cue-induced nucleus accumbens activation in alcoholics.
Methods: Non-treatment seeking alcoholics received either aripiprazole (n= 15, 13 males 2 females, mean age 26) or identical placebo (n=15, 12 males 3 females, mean age 29) for 6 days prior to an alcohol cue induced brain fMRI imaging study. In a Philips 3.0 Tesla MRI scanner, subjects were given a sip of alcohol before viewing a 12 minute randomized presentation of pictures of alcoholic beverages, non-alcoholic beverages, and two different visual control tasks. During picture presentation, changes in regional brain activity were measured in 15 transverse T2*-weighted BOLD slices. Subjects rated their urge to drink after each picture sequence. Differences in regional brain activity between viewing alcoholic beverage and non-alcoholic beverages were averaged over subjects and compared within groups and between groups.
Results: Brain activity analysis revealed increased activation for placebo-treated subjects in the right nucleus accumbens and insula (p<.05, threshold 25 voxels). In aripiprazole-treated subjects there was a complete blockade of the alcohol cue-induced activation in these areas (p<.05, threshold 25 voxels).
Discussion: In this paradigm, alcohol cue induced activation in the right nucleus accumbens and insula when subjects were taking placebo was blocked by treatment with aripiprazole. As such, aripiprazole's mechanism of action could be useful in blocking the rewarding aspects of alcohol consumption. Future study of aripiprazole in the treatment of alcoholism is warranted.

52. Functional Assessments of Stress and Reward Neurocircuitries in Post-Traumatic Stress Disorder
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Background: Anxiety and conditioned stress/fear are commonly understood to be the central features of the post-traumatic stress disorder (PTSD). A number of functional neuroimaging studies in individuals with PTSD have accordingly provided evidence of amygdala hyperreactivity to stressful stimuli as well as of hypoactive medial prefrontal cortex (mPFC) that fails to properly inhibit amygdala activity. Moreover, our recent work implicated deficits in the brain reward structures e.g., nucleus accumbens (NAc), amygdala and mPFC, subserving enjoyment of everyday events in another key PTSD characteristic, emotional numbing. Given the conspicuous overlap between the stress and reward neurocircuitries, it is in the realm of possible that altered stress and reward responsivity in PTSD are two related phenomena.
Methods: To address this issue, we employed distinct stimuli (that were previously demonstrated to reliably activate stress and reward centers in humans) in conjunction with functional magnetic reso-

nance imaging (fMRI) to determine whether PTSD is associated with altered stress and reward circuitries' activation, and whether and how these processes interact. The social stimuli were sets of aversive and pleasant visual images from the International Affective Picture System (IAPS), selected based on their scores for arousal and valence. Highly arousing and positive images were categorized as pleasant, whereas aversive images were highly arousing and negative. The sensory stimulus involved exposure to noxious vs. non-noxious thermal skin sensations (41° and 46°C, respectively). All images were acquired on a 3 Tesla Siemens Trio MR imaging system; subjects responses were analyzed using the General Linear Model in FSL.
Results: The fMRI data analyzed this far revealed that patients with PTSD (N=6), in comparison to healthy controls (N=3), displayed exaggerated amygdala activations to the aversive images in the face of diminished mPFC and NAc responses to the sensory stimuli and pleasant images.
Discussion: While we expect to have larger sample sizes at the time of the poster presentation, these findings provide a preliminary support for functional reciprocity between heightened stress reactivity and emotional numbing in PTSD (Charney, 2004), and may facilitate the emergence of new models for treatment of both clusters of these debilitating symptoms.

53. Increased 5-HT1A but Normal 5-HT2A Activity and Cerebral Blood Flow in Women Ill with Anorexia Nervosa

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Sponsor: Walter H. Kaye

Background: Disturbances of serotonin function are common in anorexia nervosa (AN). This study used positron emission tomography (PET) imaging with 5-HT receptor-specific radioligands to characterize the 5-HT1A and 5-HT2A receptors in AN. Several lines of evidence support the possibility that alterations of these receptors could play a role in AN. Disturbed 5-HT activity could contribute to altered appetite, anxious and obsessional behaviors, and extremes of impulse control in AN. We also assessed regional cerebral blood flow (rCBF) since it could contribute to physiological alterations in these underweight and malnourished subjects.
Methods: A total of 15 women who were ill with AN (7 restricting type, 8 bulimic type) were compared to 29 healthy control women (CW). PET and [11C]WAY100635 were used to assess binding potential (BP) of the 5-HT1A receptor and [18F]altanserin was used to assess postsynaptic 5-HT2A receptor BP. [15O] water and PET were used to assess cerebral blood flow.
Results: After correction for multiple comparisons, ILL AN women had a highly significant (30 to 70%) increase in [11C]WAY100635 BP in prefrontal and lateral orbital frontal regions, mesial and lateral temporal lobes, parietal cortex, and dorsal raphe nuclei compared to CW. [18F]altanserin BP was normal in ILL AN, but was positively and significantly related to harm avoidance in the lateral orbital frontal cortex ($r = .82$; $p = .004$), medial orbital frontal cortex ($r = .88$; $p = .0007$) as well as in the supragenual cingulate ($r = .68$; $p = .03$), and parietal cortex ($r = .75$; $p = .01$). Cerebral blood flow was normal in ILL AN women.
Discussion: Ill AN women had increased activity of 5-HT1A postsynaptic receptors and somato-dendritic autoreceptors and normal activity of 5-HT2A post-synaptic receptors compared to controls. Weight loss may increase 5-HT1A receptor activity in AN women, and thereby further exaggerating premorbidly existing increased 5-HT1A activity (Bailer et al., 2005). Increased activity of 5-HT1A receptor activity may help explain poor response to 5-HT medication in ILL AN. This study extends data suggesting that 5-HT function, and, in specific, the 5-HT2A receptor, is related to anxiety in AN.

54. Achieving Remission in Chronic Depression

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Background: Chronic depression is common, causes great suffering and dysfunction, and often is lethal. Previous research by our collaborative team and others has demonstrated that all studied antidepressants and a specific form of psychotherapy (the Cognitive Behavioral Analysis System of Psychotherapy [CBASP]) as monotherapies yield approximately a 50% rate of treatment "response" (ie, a 50% decrease in depressive symptoms) in patients with chronic depression. However, a combination of an antidepressant (nefazodone) plus CBASP produced an 85% response rate in completers. As a follow-up to our earlier work, NIMH funded a multi-center trial, REVAMP (Research Evaluating the Value of Augmenting Medication with Psychotherapy).
Methods: We treated chronically depressed patients open-label per an antidepressant algorithm. Analogous to the STAR*D algorithm, and designed by Trivedi, Thase, Gelenberg, and the other PIs, the algorithm evolved based on each patient's prior antidepressant history. Subjects who had not achieved "remission" by 12 weeks were assigned at random to receive: 1) next-step pharmacotherapy options per algorithm without adjunctive psychotherapy; 2) the same algorithm plus CBASP; or the medication protocol plus a supportive psychotherapy.
Results: This poster will present demographic, symptom improvement, and response/remission data from the first, open-label phase of the REVAMP study, which comprised 810 chronically depressed subjects.
Discussion: Recognition and treatment of chronic depression remains sub-optimal. Data to be presented will examine remission rates among patients treated with a best-practice protocol. Future data from this study will assess putative benefits of a specific form of psychotherapy as an adjunct to medication in this population.

55. Neuropetide Y, Calcitonin Gene-Related Peptide And CRH in Brain of Rats Bred for High Anxiety-Related Behaviors

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Background: Pathophysiology of anxiety, per se or as a symptom in other psychiatric disorders, has been only partially elucidated. A model to understand its neurobiology was developed after a decade long selective breeding of Wistar rats that resulted in lines exhibiting high (HAB) versus low anxiety behaviors (LAB). Accumulated evidence indicates that in addition to monoamines and the HPA axis hormones, other classes of biologically active compounds, notably neuropeptides play a role in brain disorders. Consequently, in this experiment we explored the possible involvement of neuropeptides in distinct brain regions of the HAB/LAB animals.
Methods: Male and female HAB and LAB rats from the colony maintained at the University of Regensburg were used. All animals were tested in elevated plus maze (EMP) at the age of 10 weeks and subjected to the Porsolt swim test 1 week later. Seven days later the animals were decapitated, the brains dissected in cryotome, tissues homogenized, and the peptides extracted and freeze-dried. Radioimmunoassays were run for neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), and corticotropin releasing hormone (CRH).
Results: BEHAVIOR: Consistently with previous results, both male and female HAB rats exhibited higher anxiety-like behavior in the EPM (assessed by % entries into and time spent on the open arm, latency to enter into the open arm) and increased depression-like behavior in the swim test (assessed by time spent struggling and floating, latency to float). NEUROPEPTIDES: NPY-like immunoreactivity (-LI) was elevated in the prefrontal and frontal cortex, cingulate and striatum in both male and female HAB compared to LAB rats. In addition, NPY-LI was increased in the entorhinal cortex and accumbens

of the male HAB compared to LAB. CGRP-LI was increased in the prefrontal and frontal cortex and hippocampus of both male and female HAB compared to LAB. CGRP-LI was also higher in the cingulate of the male HAB compared to LAB rats. The only CRH-LI difference between HAB/LAB and male/female animals was higher CRH-LI in the hypothalamus of both male and female HAB as well as LAB males compared to LAB females.
Discussion: This is the first demonstration that brain NPY and CGRP are changed in a genetic animal model of anxiety. In contrast to consistently decreased NPY expression in hippocampus of both genetic and environmental (maternal separation, chronic mild stress) models of depression, in our anxiety model no changes were observed in that region and, moreover, an increase was found in regions with significant dopaminergic and glutamatergic input and activity. CGRP, a peptide interacting with the dopaminergic system and previously found to be reactive to stress (e.g. chronic grid stress, maternal separation, ethanol withdrawal) was also increased in HAB animals. Lastly, while CRH plays a prominent role in the HPA axis dysfunction in disturbed emotionality, no changes were found in the brain CRH system in this anxiety model, confirming the dominant role of vasopressin in this anxiety model (Front Neuroendocrinol 25, 150-176, 2004). We present the first evidence that neuropeptides NPY and CGRP are altered in selected brain regions in an animal model of anxiety. Both the direction of change and the regions involved differ from those found in genetic and environmental models of depression.This implies that we may have identified some neurobiological correlates of anxiety. Such a development is likely to be useful diagnostically, contribute to better understanding of the anxiety-depression comorbidity, and may facilitate novel therapeutic approaches. SUPPORT. The Swedish MRC grant 10414 and the Karolinska Institutet

56. Habituation as a Phenotypic Marker for Major Depression

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Background: During a spontaneous episode of depression, enhanced responses to negative emotional stimuli were shown within areas of the cortico-limbic circuit that has been implicated in the pathophysiology of depression. In the present study we were particularly interested as to whether patients with remitted depression differ in their response to repeated presentation of emotionally valenced information from healthy controls.
Methods: Twenty-eight unmedicated, remitted patients with major depressive disorder (19 women) and 27 healthy control subjects (18 women) were studied during tryptophan depletion and sham depletion in a randomized, placebo-controlled crossover study. All subjects participated in two PET scanning session using 0-15 to measure cerebral blood flow (CBF). During this session, grey-scale, static face picture stimuli taken from a standard set of pictures of facial affect were presented. Because we were only interested in determining the responses to a block of emotionally expressive faces that were of a single emotion, within an individual scan all face stimuli shown over a period of 70 seconds were of the same emotional valence and intensity. Statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology) run on a Matlab 6.5 platform was used for voxel-based statistical analysis. Each subject underwent 11 blood flow scans during trytophan depletion and 11 during sham depletion. For each condition, the 11 blood flow images were realigned and a mean image generated for each subject to be used as the source for normalization. Blood Flow images were spatially normalized to a PET water template, with voxel size 2x2x2mm, in the Montreal Neurological Institute (MNI) Space. Images were smoothed using a Gaussian kernel of 8mm FWHM. The comparisons of healthy control subjects (HC) and Major Depressive Disorder subjects (MDD) were performed using a two sample t-test. The two sample t-test was also used to explore individual condition differences. Results were reported using proportional scaling to 50, and a relative threshold of 0.8. The 0.8 threshold produced an accurate mask of Brain vs. non-brain in blood flow images. Changes in blood flow were observed at a height threshold of $p = 0.001$ (corrected) as well as $p=0.01$ (corrected) and an extent threshold of 0 voxels. Clusters are reported by their size and maximum Z value (Zmax) and x, y, and z coordinates in MNI space. The MNIspace program (MSU) was used to identify regions from the Talairach coordinates provided in SPM.
Results: During TD, but not sham depletion, controls showed greater activation in response to the first presentation of unmasked neutral and sad faces relative to the second presentation, but not when viewing happy and fearful faces. Remitted depressives showed greater activation in the mediofrontal cortex, fusiform gyrus and anterior cingulate cortex during first presentation of sad and neutral faces relative to second presentation, but not when viewing happy and fearful faces. During sham depletion, remitted depressives showed greater left amygdala activation when viewing neutral and sad faces for the first time compared to repeated exposure. Controls showed greater activation to fearful faces than depressives, whereas depressives responded more to sad and neutral faces than controls.
Discussion: During TD, but not sham depletion patients with remitted depression do habituate to repeated exposure to neutral and sad faces. Similarly, during TD but not sham depletion healthy controls exhibit less activation when having repeated exposure to neutral and sad faces. The similar pattern of neural responses to repeated presentation of sad and neutral faces during TD and sham depletion in remitted depressives and controls suggests a pheotypic marker for depression.

57. Genetic and Cortico-Amygdalar Functional Connectivity Correlates of Antidepressant Treatment Response

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Sponsor: Anantha Shekhar

Background: Genotypic variations of a common polymorphism in the promoter region of the serotonin transporter (5-HTT) gene SLC6A4 (5-HTTLPR) have been reported to be associated with differential response to antidepressants. 5-HTTLPR polymorphisms can be divided into groups of high expressing (HE, LA/LA; frequency 25%), medium expressing (ME, LA/S and LA/LG; frequency: 50%) and low expressing (LE, S/S, LG/LG and LG/S; frequency 25%) genotypes. High expressing genotypes have been reported to have decreased corticoamygdalar connectivity (Heinz et al 2005; Pezawas et al 2005), decreased amygdalar activation in response to negative stimuli (Hariri et al 2002) and a possible decreased response to antidepressants. However, till present, these measures have not been measured concurrently to elucidate the relationship between genotype and neurophysiological measures that could effect antidepressant treatment response. In this study, we investigated the relationship between 5-HTTLPR genotypes and corticoamygdalar connectivity on antidepressant treatment response.
Methods: Unmedicated depressed patients (N = 10; 7F, 3M; Age: 36+9 yrs) were treated with escitalopram 10 mg po qd for 8 weeks after a 1 week single blind placebo lead in period. Functional magnetic resonance imaging (fMRI) data was collected at baseline before starting treatment using Siemens 3T magnet. Cortico-amygdalar connectivity was measured using a novel technique - correlation of low frequency BOLD fluctuations between the ventral anterior cingulate cortex (vACC) and amygdala during resting state (Anand et al 2005). Data has been collected from 10 unmedicated depressed patients. Blood was genotyped for 5-HTTLPR using previously described methods (Hu et al 2005) and patients were divided into 3 genotypic groups - HE, ME and LE.
Results: Preliminary findings of this ongoing study are presented. Genotype distribution for the 10 patients was: 3 HE, 4 ME and 3 LE. Corticolimbic connectivity was positively correlated with percent de-

crease in HDRS scores ($p < 0.06$) and this relationship became significant when genotype effect was controlled for in a two main effect ANOVA model ($p < 0.02$). Patients with the HE genotype had a greater percent decrease in HDRS scores compared to those with ME and LE genotype (92%, 60% and 74% respectively, $p < 0.01$). Patients with HE genotype also had greater corticoamygdalar connectivity compared to ME and LE genotypes but the difference was not significant between the three groups probably due to the small number of subjects studied so far.
Discussion: Preliminary data with a small number of patients is consistent with the hypothesis that at baseline both 5-HTTLPR genotype and corticoamygdalar connectivity may be used to predict antidepressant treatment response. The study is ongoing and data from more subjects needs to be collected to confirm the preliminary results. Supported by an Independent Investigator Grant (AA) from National Alliance for Research in Schizophrenia and Affective Disorders (NARSAD) and Indiana University Genomics Initiative (INGEN) fund.

58. Uncertainty-Related Brain Function in Anxiety-Disordered Adolescents

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Sponsor: Travel Awardee, ADAA, 2006

Background: Anxiety disorders are among the most common psychiatric disorders affecting children and adolescents. However, little is known about their underlying neurocognitive processes. Cognitive theories suggest that worry, a key aspect of anxiety disorders, is associated with intolerance of uncertainty and consequently, impairments in decision-making. Neuroimaging studies implicate orbitofrontal cortex, anterior cingulate cortex, and insula in both decision-making and pediatric anxiety disorders. To test this worry-uncertainty model, the present study compares neural activations in these regions in anxiety-disordered and healthy adolescents as level of uncertainty is manipulated using a decision-making task.
Methods: Sixteen adolescents with a primary anxiety disorder (ages 13- 17) and 12 age- and gender-matched non-anxious controls were recruited. Diagnostic status was determined using a semi-structured interview (ADIS-IV-C) and participants completed questionnaires assessing anxiety and intolerance of uncertainty. Functional scans were acquired at 3.0 Tesla while subjects completed a decision-making task, similar to that used by Critchley et al. (2001). Following the scan, participants were asked to rate their anxiety and certainty during the task. Mixed-effects analyses were used to detect group differences in brain activity across conditions of varying uncertainty.
Results: Behavioral findings: Anxious adolescents reported significantly greater anxiety, worry, and intolerance of uncertainty than non-anxious controls. On the decision-making task follow-up questionnaire, anxious adolescents endorsed greater anxiety ($p < .01$) and less certainty ($p = .005$) about task stimuli than controls. No significant group differences were found on measures of task performance (reaction time, accuracy). Imaging findings: Preliminary fMRI analyses testing linear contrasts across levels of uncertainty showed significant activity in anterior cingulate (BA 32) ($p < .05$, uncorrected) across groups. When individual scores on the Intolerance of Uncertainty Scale (IUS) were added to the model, the anxious group showed significant correlations between IUS scores and uncertainty-related activity in bilateral OFC (BA10/ 11) and parietal cortex (BA70/ 40) ($p < .05$, corrected). Preliminary group comparisons found greater IUS-related activity in parietal cortex and anterior cingulate in anxious adolescents as compared to controls ($p < .05$, corrected).
Discussion: Differential activations of orbitofrontal, parietal, and cingulate cortices during decision-making are suggestive of cognitive control deficits in adolescents with anxiety disorders. The implications of these findings will be discussed.

59. Orexin Antagonists as Potential Therapeutic Agents for Panic Disorder

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Background: Although the neural pathways mediating lactate-induced panic in humans are not well defined, loss of GABA inhibition in the dorsomedial hypothalamus(DMH) region produces "anxious" and panic-prone rats, i.e., having panic-like responses following 0.5M intravenous (i.v.) sodium lactate. The orexin (ORX)neurons are a critical group impacted by removal of local GABA inhibition in the DMH/PeF region that could make rats display increases in panic-like behavior. The orexin producing neurons are found only in this region, are critical for maintaining wakefulness and vigilance, contain GABAA receptor subunits, and are inhibited by GABAA receptor agonists and excited by GABA antagonists. CNS regions critical for anxiety-related responses contain extensive orexinergic fibers and receptors. Central injections of ORX produces tachycardia, hypertension and plasma concentrations of NE and epinephrine. Furthermore, specific lesions of ORX neurons reduces conditioned fear-induced tachycardia and hypertention. Therefore, the present studies investigated the role that orexin plays in panic-like states.
Methods: In the first set of experiments, all adult male Sprague-Dawley rats were anaesthetized and surgically implanted with telemetrical probes to measure cardiovascular activity. After a 3 day recovery, rats were anaesthetized and had osmotic minipumps [previously filled with the GABA synthesis inhibitor L-Allylglycine (L-AG: 3.5 nmoles/0.5µl/hr) or control isomer (D-AG) into the DMH] stereotaxically implanted unilaterally into the DMH/PeF. Later half of the l-AG and d-AG rats were challenged with i.v. infusions of lactate or saline (n=6/group). Ninety min following onset of i.v. infusions, rats were anaesthetized and perfused with 4% PFA for immunocytochemistry. In the next series of experiments, adult male Sprague-Dawley rats were anaesthetized and surgically implanted with telemetrical probes to measure cardiovascular activity. After a 3 day recovery, rats were anaesthetized and had osmotic minipumps (previously filled with l-AG) stereotaxically implanted unilaterally into the DMH/PeF. In a counter-balanced design, half of these rats received a prior intraperitoneal (i.p.) injection of an orexin receptor-1 antagonist [10-30mg/kg SB334867, in 0.2ml/100g volume DMSO] or vehicle (0.2ml/100g volume DMSO, n=6 each) prior to lactate challenge , and the effects of orexin receptor 1 blockade on lactate induced panic-like response (increased heart rate, blood pressure, respiratory rate and anxiety-like behaviors) were determined.
Results: L-AG treated rats were "anxious" (measured by social interaction test) and displayed tachycardia, increased respiration and hypertension following lactate. Immunohistochemical analysis revealed that orexin, but not melanocortin concentrating hormone-producing neurons, had increased cellular responses in the DMH of L-AG treated rats, which correlated with anxiety-like behavior ($r=0.57$, $p<0.01$). In the second study, pretreatment with orexin-1 antagonist SB334867, but not vehicle, attenuated anxiety, heart rate, respiration and blood pressure responses to lactate. In either these panic-prone rats or in another group of nonsurgical control rats (n=8), SB334867 had no effect on baseline cardiovascular measures.
Discussion: Taken together, this animal model of panic disorder suggests that hyper-responsive orexin neurons could result in increased arousal, anxiety and panic-like responses to i.v. lactate infusions. This is the first report to show a direct involvement of the orexinergic system in panic-like responses and provide pre-clinical evidence for the therapeutic potential of orexin receptor 1 antagonists as antipanic agents. Supported by NIMH RO1 MH52619 and RO1 MH065702.

60. Dimensional Predictors of Response to SRI Pharmacotherapy in Obsessive-Compulsive Disorder

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Sponsor: Travel Awardee, PMRTP, 2006

Background: With the planned introduction of DSM-V there is a worldwide movement to establish dimensional diagnostic entities. These should be consistent, replicable and backed up by a myriad of studies that include psychiatric comorbidities, functional brain imaging, genetic transmission and the response to pharmacological agents and other therapies. Such is the case for OCD where over a dozen factor analytic studies have identified six fairly consistent symptom dimensions ("symmetry/ordering", "hoarding", "contamination/cleaning", "aggressive/checking", "sexual/religious" and "somatic/miscellaneous). Previous studies have demonstrated an association between hoarding symptoms and poor response to behavioral and SRI pharmacotherapy This study sought to replicate and expand upon these previously published findings.
Methods: We recruited 164 subjects (42% male, age=35.9 + 11.0 years, prior duration of illness=14.3 + 11.7 years) seen at the Yale OCD Clinic with Axis I diagnosis of OCD. They had been seen in the 1982-1996 period for clinical treatment and/or drug trials. Each had received at least 1 of three SRI medications (Fluoxetine, Fluvoxamine, Clomipramine) for at least 8 weeks at maximum tolerated dose. Medication response was measured by CGI (Clinical Global Improvement) as assessed by a clinical rater after completion of the SRI trial. Subjects were assigned retrospectively into one of the six symptom dimensions previously mentioned based on a question that asked them to classify their own major OCD symptoms into the15 categories that came to be the major headings of the Y-BOCS. (Y-BOCS was not available for many subjects as it was introduced until 1989). Based on their answers, The Mann-Whitney U Test was used to compare CGI scores of those with and without inclusion in a dimensional category. A Bonferroni correction was used to adjust for the six separate hypotheses and set our threshold for statistical significance at $p<0.008$.
Results: We found that OCD patients with symptoms primarily in the aggression/checking domain were associated with a positive response to the SRI pharmacotherapy (Mann-Whitney U =1324, Z= -2.846, p=0.004). The rate of full response (CGI ≤2) to SRI pharmacotherapy was 69% in subjects who endorsed aggression symptoms as their primary area of concern, compared to 50% in the overall sample.
Discussion: Our findings suggest that OCD patients that symptoms lay primarily within the aggression/checking (fear of harm) domain have an increased rate of response to SRI pharmacotherapy. Despite a relatively low rate of response to SRI pharmacotherapy in OCD patients with primarily hoarding symptoms (40%), we were not able to demonstrate that hoarders had a statistically significant poorer response to SRI pharmacotherapy. This finding is likely attributable to the low number of OCD symptoms with primarily hoarding symptoms (n=5) in our sample.

61. Efficacy of Selegiline Transdermal System for Major Depressive Disorder as Measured by the HAM-D 6-item Bech Subscale

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Sponsor: Donald S. Robinson

Background: The Hamilton Depression Rating Scale (HAM-D) 6-item Bech subscale (Bech-6), a direct measure of core depressive symptoms, may be superior to total HAM-D score in detecting antidepressant effiacy of drugs.1-3 Selegiline transdermal system (STS), a monoamine oxidase inhibitor with a unique pharmacokinetic and pharmacodynamic profile, is efficacious for treatment of major depressive disorder (MDD).4-6 The objective of this analysis is to assess the antidepressant efficacy of STS, using the Bech-6, across all short-term (6 to 8 weeks) placebo-controlled efficacy trials conducted with STS in adult outpatients with MDD and to compare treatment outcomes with results employing the HAM-D and MADRS total score.
Methods: In each of 5 randomized, double-blind, placebo-controlled trials (4 STS fixed-dose trials [6 mg/24 hr] and 1 STS dose-titration study [6 mg/24 hr to 12 mg/24 hr]) change in the Bech-6 total score from baseline to treatment endpoint was assessed in STS and placebo treatment groups. HAM-D and MADRS data were analyzed separately for each individual study and also pooled for meta-analysis. Results: In 4 of 5 individual studies, mean improvement from baseline in Bech-6 scores was statistically significant ($P<0.05$) for the STS treatment groups compared with placebo. Meta-analysis of all 5 studies also demonstrated a highly significant treatment effect for STS ($P<0.001$) on core depressive symptoms. Based on total HAM-D score, 2 of 5 trials met the a priori endpoint and 1 trial was considered supportive of efficacy ($P=0.06$). All 3 of these studies met the secondary efficacy endpoint for MADRS total score.
Results: In 4 of 5 individual studies, mean improvement from baseline in Bech-6 scores was statistically significant ($P<0.05$) for the STS treatment groups compared with placebo. Meta-analysis of all 5 studies also demonstrated a highly significant treatment effect for STS ($P<0.001$) on core depressive symptoms. Based on total HAM-D score, 2 of 5 trials met the a priori endpoint and 1 trial was considered supportive of efficacy ($P=0.06$). All 3 of these studies met the secondary efficacy endpoint for MADRS total score.
Discussion: Four of 5 placebo-controlled efficacy trials show significant STS treatment effects for core depressive symptoms of MDD, as measured by the Bech-6. The 17-item HAM-D score, which includes 3 insomnia items, appeared to be less sensitive in detecting antidepressant effect than the Bech-6 and MADRS. These depression rating scales may be superior to the 17-item HAM-D for assessing efficacy of non-sedating antidepressants such as STS. These results confirm that the therapeutic benefit of STS is attributable to intrinsic antidepressant treatment effects. References: 1 Bech P, et al. Quantitative rating of depressive states. Acta Psychiatr Scand 1975;51:161-170. 2 Faires D, et al. The responsiveness of the Hamilton Depression Rating Scale. J Psychiatr Res 2000;43:3-10. 3 Entsuah R, et al. A critical examination of the sensitivity of unidimensional subscales derived from the Hamilton Depression Rating Scale to antidepressant drug effects. J Psychiatr Res 2002;36:437-448. 4 Bodkin JA, Amsterdam,J.D. Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. Am J Psychiatry 2002; 159(11):1869-1875. 5 Amsterdam JD. A double-blind, placebo-controlled trial of the safety and efficacy of selegiline transdermal system without dietary restrictions in patients with major depressive disorder. J Clin Psychiatry 2003;64:208-214. 6 Feiger AD, et al. Selegiline transdermal system for the treatment of major depressive disorder: an 8-week, double-blind, placebo-controlled, flexible-dose titration trial. J Clin Psychiatry 2006; in press.

62. Identifying Neural Substrates Mediating Chronic Fluoxetine Effects in the Novelty-Induced Hypophagia Test

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Sponsor: Rita J. Valentino

Background: Despite their widespread use for treating depression and anxiety, relatively little is known about the mechanisms through which chronic antidepressants are effective. The novelty-induced hypophagia test (NIH) measures the suppressed consumption of a familiar food by exposure to a novel environment. This is one of few animal behavior tests that are responsive to both acute anxiolytic and to chronic, but not acute, antidepressant treatments. Although nu-

merous neuroadaptations result from chronic antidepressant treatments, the critical neural circuitry mediating repeated antidepressant treatment efficacy remains uncertain. The goal of the present experiment was to identify the neural substrates underlying the effects of chronic fluoxetine treatment using the NIH test.
Methods: Pair-housed male Sprague-Dawley rats were trained to eat graham cracker crumbs individually in their home cage (15 min/day). After daily intakes stabilized, animals were implanted with osmotic minipumps delivering either vehicle or fluoxetine (5 or 20 mg/kg/day). The animals were then left undisturbed in the colony for 23 days. In order to reacquaint animals with the palatable food and procedures, animals were subjected to 5 days of home cage feeding, with the last day serving as the home cage test. The next day, novelty-induced suppression of feeding was assessed by giving animals access to the familiar palatable food (graham cracker crumbs) in a novel environment. Ninety minutes after the start of the final feeding session, animals were given a high dose of pentobarbital and transcardially perfused with paraformaldehyde to prepare the tissue for immunohistochemical analysis of c-fos expression.
Results: Exposure to novelty decreased food intake and increased latencies to eat in the novel cage compared to the home cage in vehicle-treated animals. Chronic treatment with fluoxetine mitigated these effects. Expression of c-fos was compared between vehicle-treated animals sacrificed after a home-cage test and vehicle- or fluoxetine-treated animals exposed to the novel environment in 22 brain regions. This analysis revealed significant treatment effects for c-fos expression in the anterior nucleus accumbens Shell (aAcbSh), cingulate cortex (Cg), dorsal CA2 (dCA2), lateral bed nucleus of the stria terminalis (BSTL) and piriform Cortex (Pir). Novel cage exposure increased c-fos expression in the Cg and fluoxetine treatment returned c-fos expression to home cage levels. Regression analyses revealed that decreased food intake in the novel cage was associated with a greater number of c-fos positive cells in the Cg ($r = -0.49$). In contrast, the effects of c-fos in other regions may have been driven by exposure to drug since there was no relationship between c-fos expression and exposure to novelty.
Discussion: The present results implicate the Cg in chronic antidepressant treatment effects in the NIH test. In particular, the Cg was activated in response to exposure to novelty and this effect was prevented by chronic antidepressant treatment. This finding bears striking similarity to those of depressed patients where over-activation of the cingulate is observed and inactivation alleviates symptoms of depression. Taken together these findings support a putative causal role of the cingulate cortex in the ability of antidepressants given chronically to alter behavior.

63. The Persistence of the Placebo Response

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Sponsor: Walter Brown

Background: In order to examine the widely held assumption that when depressed patients treated with placebo improve, the improvement is transient, we examined relapse rates of patients assigned to placebo during the acute phase of an antidepressant clinical drug trial who continued to receive placebo treatment during the continuation/ maintenance phase of the study.
Methods: Through a combination of relevant literature reviews and a Medline search, we identified six published randomized, double-blind, placebo-controlled clinical trials which had an acute phase followed by a continuation/ maintenance phase in which the patient remained on the same treatment of antidepressant (AD) or placebo for longer than 12 weeks. We calculated percentages for the number of patients entering the continuation/ maintenance phase, and the number of patients entering the continuation/ maintenance phase who subsequently relapsed.
Results: Our combined analysis of the six studies was composed of a total of 2591 patients, of which 53% (n=907) of the antidepressant group and 38% (n=334) of the placebo group were responders to acute treatment and continued into the continuation/ maintenance phase of the study. Based on the total number of patients entering the continuation/ maintenance phase in all six of the studies examined, 7% of AD responders and 22% of placebo responders met relapse criteria during this phase.
Discussion: Although significantly more patients relapsed in the continuation/ maintenance phase of the trials on placebo than antidepressant, 4 out of 5 placebo responders entering the continuation/maintenance phase of the trial stayed well. This suggests that placebo responders in randomized double-blind clinical trials who continue into the extension phase of the study on the same treatment are likely to remain well for more than 12 weeks. These data suggest that the widely held and stated belief that the placebo response is short-lived seems based more on intuition and perhaps wishful thinking than on the extant data.

64. Relationship Between Depression Severity Entry Criteria and Antidepressant Clinical Trial Outcomes

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Sponsor: Harbans Lal

Background: Antidepressant clinical trials are plagued with unpredictable results and high failure rates. It has been suggested that factors such as inclusion of more severely ill depressed patients, use of flexible dosing and influencing sex distribution in the trial sample may enhance antidepressant-placebo difference and in turn provide a more favorable outcome for the test antidepressant. Based on this suggestion, some of the newer antidepressant trials are being designed to include more severely ill depressed patients by requiring a higher threshold of depressive symptoms. In the current study, we assessed if increasing the minimum pre-randomization HAM-D score in order to enrich the test sample of depressed patients would affect antidepressant trial outcome.
Methods: Using the efficacy sections of FDA SBA reports, we examined outcome data from 51 clinical trials (11,270 depressed patients) evaluating ten investigational antidepressants. We recorded the following critical variables: trial identifier, duration of trial in weeks, dosing schedule (fixed vs. flexible), number of treatment arms, dose administered for each treatment condition, number of patients in each condition, minimum HAM-D screen criteria, mean pre-randomization scores, changes in HAM-D scores at last visit, and trial outcome. Trials were grouped into four categories based on the minimum pre-randomization HAM-D score for each trial. We conducted ANOVA and chi square analyses to determine if differences existed between the four categories in pre-randomization scores, dosing schedule, and trial outcome as well as the above mentioned variables that could potentially act as confounds in our analysis.
Results: The antidepressant clinical trials requiring the highest mean total pre-randomization HAM-D score (>20 HAM-D 17) had the lowest frequency of positive outcomes (20%), compared to the other three lower category groups (49%, $\chi 2 = 4.04$, df =1, $p = 0.04$). Paradoxically, high entry criteria requirements failed to reliably increase actual mean total pre-randomization HAM-D scores. A larger magnitude of symptom reduction was seen among patients assigned to placebo in the antidepressant clinical trials with the highest entry criteria for severity of depressive symptoms. Overall, the mean total pre-randomization HAM-D scores as well as use of flexible dosing were associated with higher rates of positive outcome.
Discussion: In summary, requiring higher pre-randomization depressive symptoms was not associated with an increased rate of favorable outcomes among these 51 antidepressant trials. In fact, our results suggested a high entry criteria was associated with greater

frequency of failure, in part due to little correlation between entry criteria requirement and actual mean baseline total HAM-D scores. In other words, antidepressant clinical trials with the highest requirement had the lowest mean total HAM-D scores at randomization and also the largest magnitude of symptom reduction with placebo.

65. COMT Genetic Variation and Depression: Gene-Environment Interaction and HPA Axis Correlates

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Variation in the gene for Catechol-O-Methyltransferase (COMT), has been associated with a number of psychiatric disorders, including psychotic, anxiety, and affective disorders. Gene-environment interactions in predicting depression have been demonstrated for a polymorphism in the promotor region of the serotonin transporter (SERT), but gene-environment interactions with COMT have not been reported. We tested the hypothesis that allelic variation in the functional COMT Val158Met polymorphism (rs4680) would interact with childhood maltreatment in the prediction of lifetime history of depressive disorders.
Methods: One hundred thirty-one European-American adults, 61 with a depressive disorder, 70 with no Axis I diagnosis, completed the Childhood Trauma Questionnaire and provided blood samples for DNA extraction and genotyping.
Results: COMT genotypes were in Hardy-Weinberg Equilibrium and did not differ with regard to age or sex. A logistic regression model predicting depressive diagnoses was significant (chi sq=33.2, df=5, p<.001), and explained 30% of the variance. There was a significant effect of COMT genotype (B= -4.4, p=.005) and a significant interaction between genotype and reports of childhood maltreatment (B=0.56, p<.01). Specifically, individuals with the Met158/Met158 genotype were more likely to have a depressive disorder irrespective of childhood maltreatment, whereas those with the Val allele had an increase in the likelihood of depressive diagnosis when they reported a history of maltreatment. In addition, a subset of the sample (N = 53) underwent a standardized psychosocial stress challenge, the Trier Social Stress Test (TSST), during which cortisol reactivity was assessed. In a repeated measures general linear model predicting cortisol response to this test and controlling for age and sex, there was an effect of COMT genotype (F(1,48)=4.35, p<.05) such that individuals with the Met158/Met158 genotype had lower cortisol responses to the TSST compared to those with the Val allele.
Discussion: These findings provide support for the view that variations in the COMT genotype may predispose individuals to maladaptive stress responding and depression.

66. Placebo-Controlled Trial of Risperidone Augmentation for SSRI-Resistant Civilian PTSD

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Sponsor: R. Bruce Lydiard

Background: The prevalence of Posttraumatic Stress Disorder (PTSD) has been estimated at 9%1 to 12.3% of the general population, clearly indicating that the disorder is a major public health concern. Treatment of PTSD with pharmacotherapy is promising. Two serotonin reuptake inhibitor agents (sertraline and paroxetine) have received FDA indications for the treatment of PTSD. In spite of these promising findings, it is generally agreed that the response to medication has been modest. Therefore, strategies to bolster the response to antidepressant medications are warranted. The atypical antipsychotics have recently been shown to be an effective adjunct to treatment with SSRIs in treatment resistant depression. In a recent study in combat veterans with PTSD, there was significant improvement in symptoms of PTSD with the addition of risperidone to antidepressant treatment in individuals who were experiencing psychotic symptoms. Risperidone as an adjunct to antidepressant treatment has not been tested in a double-blind placebo-controlled trial in civilians with PTSD.
Methods: The primary objective is to compare the response of civilians with PTSD currently receiving sertraline without an optimal response randomly assigned to receive risperidone augmentation or matching placebo. In Phase I, subjects were treated for eight weeks with open label sertraline. Those who did not remit, defined as a 70% decrease in PTSD symptoms as measured by the Clinician administered PTSD Scale CAPS, were entered into Phase II. In Phase II, subjects remained on the sertraline and were randomly assigned to have their medication augmented by risperidone or matching pill placebo for 8 weeks. Symptoms of PTSD, depression, quality of life, resilience, and psychotic symptoms were measured prospectively throughout the 16-week study. Measures included: Interviews: Mini International Neuropsychiatric Interview (MINI) - for assessment of psychiatric diagnoses; Clinician Administered PTSD Scale (CAPS) - to assess symptoms of PTSD.; and the Positive and Negative Symptoms Scale (PANSS) - to assess psychotic symptoms. Self-Report Measures: Davidson Trauma Scale (DTS) - assesses the 17 diagnostic symptoms of PTSD; Beck Depression Inventory (BDI); PTSD Symptom Scale – Self- Report (PSS-SR). Therapist Measures: Clinical Global Improvement Scale (CGI).
Results: 91 patients were consented and screened, 50 completed baseline assessment of which 45 were eligible to enter phase I; 34 completed Phase I (open label); 24 were randomized to Phase II; and 18 completed Phase II thus far. The blind will be broken in October when the last patients will complete, so means are reported for the entire sample here but will be for each group when presented. On average, patients were 33.2 (SD 10.9) years of age, 83% female, 67% Caucasian, 39% received some college, 72% were never married, with 78% having comorbid major depression. CAPS total scores decreased significantly over time, from a mean of 76.17 (SD 14.21) at Visit 2 (Phase I open label baseline), to 60.44 (SD 18.01) at Visit 5, to 59.11 (SD 18.05) at Visit 7 (Phase I endpoint), to 47.28 (SD 21.76) at Visit 10, finally to 35.83 (SD 19.39) at Visit 12 (Phase II randomized endpoint). PANSS General scores also decreased significantly over time ($p < .05$) in Phase II.
Discussion: The addition of an atypical antipsychotic appears to have resulted in decreased PTSD and psychotic symptoms in this sample of PTSD civilians gathered at three different sites.

67. Glutamate Signaling Proteins in Major Depression

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Sponsor: Garth Bissette

Background: Accumulating evidence suggests dysfunction of the glutamate signaling system in major depressive disorder. Previously, we observed elevated levels of the NR2C subunit of the N-methyl-D-aspartate receptor (NMDAR) and reduced levels of neuronal nitric oxide synthase (nNOS), an intracellular mediator of NMDAR activation, in the noradrenergic locus coeruleus in depressed subjects (Karolewicz et al. 2004, 2005). Despite significant evidence for abnormal glutamatergic signaling in depression, the molecular mechanisms that contribute to these abnormalities at the level of glutamate synapses have not yet been characterized. The aim of the present study was to investigate potential abnormalities in the glutamate signaling molecules in brain regions associated with severe depression such as the amygdala and hippocampus.

Methods: Tissue samples containing the lateral nucleus of amygdala, and hippocampal dentate gyrus were obtained from 10 - 12 matched pairs of depressed subjects and healthy controls. Subjects were matched for age, sex, brain pH, and postmortem interval. Changes in concentration of NMDAR subunits NR1 and NR2 as well as intracellular associated proteins, neuronal nitric oxide synthase (nNOS) and postsynaptic density protein (PSD-95), that might occur in depression were assessed by immunoblotting. Additionally, in the amygdala we measured the level of glutamate metabolizing enzymes, glutamine synthetase (GS), and glutamic acid decarboxylase (GAD-67).
Results: The overall amounts of NR1, nNOS, GS, and GAD-67 were unchanged in subjects diagnosed with depression as compared to controls. Amounts of PSD-95 were significantly higher in the amygdala (+130 %, $p<0.05$) and hippocampal dentate gyrus (+34%, $p<0.05$). The level of NR2A subunit was elevated in the amygdala (+51%), but not in the hippocampus in depressed subjects as compared to controls.
Discussion: Our data indicate that glutamatergic signaling at the NMDAR is abnormal in depression. Significantly higher levels of NMDAR subunit and its associated anchoring protein, PSD-95, may represent an adaptive response to decreased synaptic activation by glutamate. This hypothesis is in agreement with postmortem and neuroimaging findings of altered glutamatergic transmission in depression. Further studies of the glutamatergic signaling system may lead to the development of novel therapeutics for the treatment of depressive disorder. The project described was supported by Grant Number RR17701 from the National Center for Research Resources (NCRR), and MH63187, MH46692, MH46694.

68. Elevated Plus Maze Behavior in Meprin Beta Knockout Mice

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Sponsor: Thomas W. Uhde

Background: Discovered in the 1980's, meprins are cell-surface and secreted mammalian metalloproteases that are primarily expressed in kidney and intestine. One role of meprins is to hydrolzye proteins such as fibronectin and biologically active peptides such as bradykinin, neuropeptide Y and gastrin. Meprins have been linked to inflammatory diseases of the kidneys and intestines, and are found in leukocytes and certain cancer cells. Anxiety-like behavior has been previously noted in meprin β knockout mice, but has not been systematically studied. This is the first test of fear induced anxiety-like behavior in meprin β knockout (βKO) mice.
Methods: Fourteen adult male βKO mice on a C57BL/6 background were tested in the Elevated Plus Maze (EPM). Twelve adult male C57BL/6 (C57) mice (Jackson Laboratory) served as controls. The EPM, a validated test to measure fear-/anxiety-like behavior in laboratory animals, was employed to test the meprin β KO in this study. The EPM (San Diego Instruments, San Diego, CA) was made of white plastic with lightly textured arms. The 2 open arms (30.5 x 5.1 cm) had a small ledge, while the closed arms were the same size as the open arms but with 15.2 cm high walls. The 5.1 x 5.1 cm central square connected the opposite open and closed arms, thus forming a plus-sign. The maze was elevated 38.1 cm above the floor. Each mouse was placed in the maze for a 5 minute trial and videotaped for later analysis. Investigators measured both time spent and number of entries made in open versus closed arms of the maze, as well as total distance traveled.
Results: The results showed that meprin βKO mice made significantly more entries into (βKO=6.00+/-3.06, C57=4.00+/-1.76) and spent a significantly higher percentage of time in (βKO=17.52%, C57=8.25%) the open arms of the EPM as compared with C57 mice ($t24=2.65$, $p=0.01$; $t24=1.99$, $p=0.05$, respectively). The total mean distance traveled by meprin βKO mice, however, was found to be similar to that traveled by C57 mice (βKO=10.5 m, C57 =10.1 m). Consequently, the differential number of entries into and time spent in the open arms as a function of mouse strain cannot be attributed to differences in activity level between these two groups. Therefore, these results indicate that meprin βKO mice exhibited a decrease in behavioral inhibition on the open arms of the EPM, but were not hyperactive when compared to their C57 counterparts. Open arm entry, then, was not driven by hyperactivity.
Discussion: The present study is the first to examine anxiety- and fear-like behavior in meprin βKO mice. Meprin βKO mice were previously noted to appear "anxious" when handled in blood pressure experiments. The EPM demonstrated reduced anxiety- and fear-like behavior in these mice, indicating that there are behavioral differences associated with the meprin β gene, Additional behavioral testing in meprin βKO mice is warranted to explore the phenotype. Other animal models of anxiety (e.g. social interaction, open field and startle) could aid in this pursuit. (Supported by NIH Grant DK19691).

69. The Effects of Pentazocine, A Kappa Agonist, in Mania

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Background: Results both of animal and human studies suggest that modulation of kappa opiate receptors (KOR) can alter mood. In animal models, direct activation of KOR causes depressogenic effects, while blockade of KOR has antidepressant-like effects. Notably, the effects of current antipsychotic drugs, which are potent antimanic agents, may be mediated, in part, through an increase in the activity of dynorphinergic neurons resulting in the release of dynorphin, the endogenous agonist at KOR. Opiates, which largely target mu opiate receptors, have been given in the past to patients with bipolar disorder, but specific agonists of KOR have not been tested as treatments for mania. No specific kappa agonist is currently approved for human use. However, the analgesic agent pentazocine is predominantly active at KOR, at which it is a partial agonist. It has lower affinity and weaker effects at mu opiate receptors and sigma receptors. We administered pentazocine to bipolar patients to test the possibility that kappa agonists would reduce the symptoms of mania.
Methods: Ten inpatient subjects with a primary diagnosis of bipolar mania and a Young Mania Rating Scale (YMRS) score of greater than or equal to 14 were enrolled in the study. The study was designed as an open-label cumulative-dosing trial. The three-day study consisted of pre-treatment, treatment, and post-treatment days with subjects rated in the morning on all three days and multiple times on the day of treatment. On the treatment day, an initial 50mg of pentazocine was given by mouth. A second dose was given two hours following the initial dose. Assessment of manic symptoms by YMRS were made daily at the same time in the morning on all three days of the study. YMRS and DSMIV criteria were used as the basis for constructing a scale to detect acute changes in manic symptoms (the Mania Acute Changes Scale or MACS) The MACS was given along with the YMRS in the morning on all three days of the study. In addition, the MACS was given hourly for 6 hours starting with the first dose of pentazocine.
Results: No subject experienced significant side effects by clinician ratings or self-report. All subjects experienced an improvement in manic symptoms, as measured by total score on the MACS, following the administration of pentazocine. Symptoms of mania were reduced one hour after each dose, 44% after the first dose and 41% after the second dose. These reductions were statistically significant ($F=3.69$, $p=0.01$). No subject experienced exacerbation of psychotic symptoms or complained of dysphoria. Ratings of sedation were not significant. Over the three days of the study, YMRS scores improved daily with no evidence of a rebound in symptoms after treatment.
Discussion: Administration of pentazocine was associated with a transient, but substantial and statistically significant, reduction in

manic symptoms. No adverse effects, including psychotomimetic effects or sedation, were reported. Limitations in interpreting these results arise from the open nature of the trial, the small number of subjects, and the limited number of doses studied. Nonetheless, these initial results are promising and support further investigation into the mood-regulating properties of kappa-opiate agents.

70. Do Antidepressants Differ in How Well They Treat Major Depression with Coexisting Anxiety? A Systematic Review of the Evidence

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Sponsor: Robert N. Golden

Background: MDD is frequently associated with concurrent anxiety. If certain antidepressants can more successfully treat such a depression, or if they can mitigate the specific concurrent anxiety symptoms, these agents might be preferred choices. In a report commissioned by the Agency for Healthcare Research and Quality, we systematically reviewed the evidence addressing two questions: Q1: Do medications differ in their efficacy and effectiveness in treating the depressive episode? Q2: Do medications differ in their efficacy and effectiveness in treating the accompanying symptoms?
Methods: We searched MEDLINE®, Embase, The Cochrane Library, and the International Pharmaceutical Abstracts from 1980 to February 2006. We manually searched reference lists of pertinent review articles and letters to the editor and explored the Center for Drug Evaluation and Research database to identify unpublished research submitted to the US Food and Drug Administration. We included double-blinded, randomized controlled trials of good or fair internal validity. Two persons independently reviewed abstracts and full text articles. We used a structured data abstraction form to ensure consistency in appraisal and data extraction.
Results: Of 878 articles identified and reviewed, 13 met our inclusion criteria and were included in our synthesis. For Q1, evidence from 6 fair-quality head-to-head trials and 1 fair-quality placebo-controlled trial suggests that antidepressant medications do not substantially differ in antidepressive efficacy for patients with MDD and anxiety symptoms. Trials found no substantial differences in efficacy between fluoxetine, paroxetine, and sertraline; sertraline and bupropion; and sertraline and venlafaxine. One trial found statistically significant superiority of venlafaxine over fluoxetine. The strength of evidence is moderate. For Q2, evidence from 10 fair-quality head-to-head trials and 2 fair-quality placebo-controlled trials provide evidence that antidepressant medications do not differ substantially in efficacy for treatment of anxiety associated with MDD. Trials found no substantial differences in efficacy between fluoxetine, paroxetine, and sertraline; sertraline and bupropion; sertraline and venlafaxine; citalopram and mirtazapine; paroxetine and nefazodone. One trial found that venlafaxine was statistically significantly superior compared to fluoxetine.The strength of evidence is moderate.
Discussion: For patients with high anxiety associated with MDD, we found no difference in patients' depression treatment response by either antidepressant class or specific medication. Although all the included studies identified a high anxiety group, the definitions employed by investigators varied markedly. In addition, for patients with anxiety symptoms associated with depression, we found no identifiable difference in anxiety response by either antidepressant class or specific medication. The current evidence suggests that improvement in both depressive and anxiety symptoms are likely with adequate dosing of antidepressant treatment, but evidence of clear benefit for one antidepressant over another is lacking. This study was conducted by the RTI/UNC Evidence-based Practice Center under contract to the Agency for Healthcare Research and Quality, Contract No. 290-02-0016, TO #7, Rockville, Maryland. The authors of this article are responsible for its contents, including any clinical or treatment recommendations. No statement in this article should be construed as an official position of the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

71. Neural Connectivity in Children with Bipolar Disorder: Impairment in the Face Emotion Processing Circuit

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Sponsor: Past Travel Awardee, ADAA, 2005

Background: Pediatric bipolar disorder (PBD) is a highly debilitating illness associated with impaired identification of emotional face expressions and related social deficits. Neuroimaging studies find that PBD is characterized by amygdala abnormalities, i.e. volume reduction and hyperactivation during deficient face emotion processing. However, brain regions are not isolated entities but instead form highly inter-connected neural networks. The current study examined possible perturbed amygdala functional connectivity with other brain regions. Such evidence would implicate a distributed neural circuit in the pathophysiology of PBD, and would further elucidate the neural mechanisms associated with PBD face emotion misinterpretation.
Methods: Subjects were 33 PBD (ages 9-18 years) and 24 healthy controls who were age, gender, and IQ-matched. PBD subjects had strictly defined DSM-IV bipolar disorder, including a history of at least one episode (> 4 days) of hypo/mania including euphoric mood. Participants completed a functional Magnetic Resonance Imaging (fMRI) task of face emotion identification. Subjects' attention was directed to faces of varying emotional and nonemotional expressions while subjects were asked to rate emotional (hostility, fearfulness) and nonemotional (nose width) aspects of the faces. Rapid event-related fMRI BOLD signal data on a 3T scanner was collected. Functional connectivity was measured by examining the coupling of activation between the left amygdala and each voxel across the brain.
Results: Compared to healthy subjects, PBD subjects had significantly reduced connectivity between the left amygdala and four regions: left superior temporal gyrus (t=3.31, p=.001 uncorrected), right fusiform gyrus (t=3.27, p=.001 uncorrected), right posterior cingulate/precuneus (t=3.34, p=.001 uncorrected), and left perisylvian fissure (t=3.40, p=.001 uncorrected). These regions have been implicated previously in processing facial expressions and social stimuli. There was a significant association between reduced connectivity in PBD subjects and more negative misperceptions of face emotions (r=-.45, p=.009), but not in controls. Results were not related to PBD patients' mood state or comorbid diagnoses.
Discussion: PBD youth exhibit deficient neural connectivity between the amygdala and temporal association cortical regions previously implicated in face processing. These results expand on prior evidence of volumetric and functional amygdalar deficits in PBD youth. Our results show that impaired labeling of face emotions in PBD youth relates to dysfunction in a distributed neural network connecting the amygdala and regions critical to processing faces and evaluating their social significance. These results may begin to clarify the neural correlates of social deficits in PBD youth and further elucidate the pathophysiology of the disorder.

72. Escitalopram in the Treatment of Diabetic Patients with Major Depressive Disorder

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Background: The incidence of depression is greater in patients with diabetes compared with the general population. Furthermore, de-

pression has been associated with decreased glycemic control and a higher rate of complications of diabetes. The present trial examined the efficacy and safety of escitalopram in the treatment of depression in diabetic patients.
Methods: Outpatients (aged 18-80 years) with Type I or Type II Diabetes Mellitus and DSM-IV diagnosed MDD (Montgomery-Asberg Depression Rating Scale (MADRS) total score ≥22) were randomized to 12 weeks of double-blind, flexible-dose treatment with escitalopram 10-20 mg/day (N=83) or placebo (N=83). The primary efficacy parameter was change from baseline to week 12 in MADRS total score using the last observation carried forward (LOCF) approach.
Results: Treatment was completed by 83% of escitalopram-treated patients and 86% of patients in the placebo group. Mean baseline MADRS total scores were 28.9 and 30.2 for the escitalopram and placebo groups, respectively. The change from baseline to week 12 in MADRS total score (LOCF) was clinically meaningful (LSMD=-2.14) but was not statistically significant (p=0.196) given the relatively small sample size. However, statistically significant improvements were observed in the completer population (observed case (OC) analyses) at week 8 (LSMD=-3.52), week 10 (LSMD=-4.50), and week 12 (LSMD=-3.35). Post-hoc analysis revealed a statistically significant effect of escitalopram treatment in the subset of moderately depressed patients (baseline MADRS total score <30) at weeks 10 and 12 (LOCF, OC; p<0.05). Discontinuation rates due to adverse events were 4% in the escitalopram group and 2% in the placebo group. Treatment-emergent adverse events occurring at a frequency of ≥10% in either escitalopram-treated patients or in the placebo group were diarrhea, headache, nausea, and upper respiratory tract infection.
Discussion: Escitalopram treatment produced clinically meaningful improvements in patients with diabetes and was safe and well-tolerated. In patients who completed the study, escitalopram provided greater benefit in reducing symptoms of depression.

73. fMRI Brain Activation Associated with Mood State Differences in Frequently Relapsing Bipolar Patients Treated with Long-Acting Injectable Risperidone

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Background: Frequently relapsing bipolar disorder represents a significant clinical treatment challenge. In part, this is related to a poor understanding of the neurophysiology of mood states in these patients. In order to address this, we studied frequently-relapsing bipolar patients using fMRI while they participated in an ongoing clinical study of the long-acting injectable (LAI) form of risperidone (Risperdal Consta®).
Methods: At baseline bipolar patients with mania (YMRS > 13) were compared to 10 nonmanic patients (YMRS ≤ 12) while performing the degraded-stimulus version continuous performance task (CPT-DS) and receiving treatment as usual (TUA). Additionally, associations between changes in YMRS and depression ratings (MADRS) and regional fMRI brain activation were examined in all patients (N=19) while receiving LAI-risperidone treatment added to (TUA). MRI data were 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 standard methods in AFNI.
Results: At baseline, patients with mania exhibited significantly greater activation in the left medial frontal (BA 10) and left anterior cingulate (BA 32), whereas nonmanic patients exhibited greater bilateral superior temporal/insula and left, mid-occipital and caudate activation. Impulsive responding on the CPT-DS was associated with increased subgenual cingulate (BA 25) and amygdala activation in the nonmanic patients. Over time while on LAI-risperidone treatment, YMRS scores were inversely correlated with activation in the left middle frontal gyrus (BA 10) and left anterior cingulate (BA 24/32). Depression scores (MADRS) across time were inversely correlated with medial and middle FG (BA10), subgenual cingulate (BA 25), and posterior attentional areas bilaterally.
Discussion: Patterns of fMRI brain activation in these frequently-relapsing bipolar patients receiving LAI-risperidone added to TUA suggest specific associations between mood symptoms and activation of anterior limbic brain areas. Treatments that target these activation abnormalities may demonstrate improved management of the symptoms of rapid-cycling bipolar disorder. Funding provided by Janssen, L.P.

74. Comorbid Substance Use Disorders in Depressed Outpatients: A Secondary Analysis of the MAST and DAST in the Texas Medication Algorithm Project

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Sponsor: Past Travel Awardee, PMRTP, 2005

Background: Co-occurring substance use and depressive disorders present a difficult set of challenges to providers and researchers interested in developing appropriate treatments for this population. Estimates of the prevalence of substance use disorders (SUD) in patients with major depressive disorder (MDD) are mostly derived from population based surveys of substance abuse and vary considerably. It is unclear how antecedent or chronic MDD interacts with a co-occurring SUD. Recently published data from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) provides a relevant estimate of the burden of substance use disorders in a large, community-based, multi-center trial designed for persons seeking treatment for major depression. In STAR*D, baseline clinical characteristics of 1484 patients were examined and 28% were found to have symptoms consistent with an active SUD (Davis et al, 2005). In addition, the study also found that persons with major depression and a concurrent SUD had earlier onset of depression (P=0.014), significantly higher rates of suicide attempts (P=0.014), hypersomnia (P=0.006), anxious mood (P=0.047) and suicidal ideation (0.036) compared to MDD alone (Davis et al, 2005). The Texas Medication Algorithm Project (TMAP), which in part led to the development of STAR*D, involved the development of medication and psychosocial treatment algorithms for MDD. Unlike STAR*D, TMAP collected a more specific set of data on substance use comorbidity in the form of the Michigan Alcohol Screening Test (MAST) and Drug Abuse Screening Test (DAST) and other supplemental substance use information. TMAP may offer a more detailed description of co-occurring SUD using validated screening instruments. Further analyses of the interaction between SUD and depressive symptom clusters may also be examined.
Methods: Data from the MDD module of TMAP were used to compare patients based on substance use. All treatment groups (ALGO and TAU) were combined for this analysis (n=542). Only baseline data were used. Patients were classified based on the MAST and the DAST where a MAST of 5 or more indicates problem drinking and a DAST of 6 or more indicates moderate level drug abuse. Four groups were created: Alcohol abuse only, drug abuse only, both alcohol and drug abuse, neither alcohol nor drug abuse. In this sample, p-values compare each abuse group to the "Neither" group except in the within substance use comparisons.
Results: Among depressed outpatients, 28% screened positive for concurrent problem drinking, only 2% screened positive for moderate level drug abuse and 6% screened positive for both. Depressed outpatients with drug abuse and both alcohol and drug abuse were significantly younger than the depressed outpatients with neither (p=0.01 and 0.005, respectively). Depressed outpatients of all racial groups were more likely to abuse alcohol than other substances (p<0.05 for all groups). Outpatients with both alcohol and substance

use problems had an earlier age of onset of major depression vs. neither (p=0.04). When compared across substance use categories, those with drug only (p=0.03) and both alcohol and drug abuse (p=0.04) had an earlier onset of MDD vs. the alcohol only group.
Discussion: These data highlight the significant burden of problem drinking and combined alcohol and drug use in depressed outpatients. The association of problem drinking and moderate level substance abuse with earlier age of onset of MDD suggests a potentially longer and severe course of MDD in clients with co-occurring SUD. The role of low-level non-alcoholic drug abuse in depressed patients requires further investigation.

75. Efficacy of a Protein Kinase C Inhibitor in the Treatment of Acute Mania: A Double-Blind, Placebo-Controlled Study
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Sponsor: Husseini Manji

Background: Elucidation of the mechanism(s) by which lithium stabilizes an underlying dysregulation of limbic and limbic-associated function also offers the potential to delineate the underlying pathophysiology of bipolar disorder; however, a major problem inherent in neuropharmacologic research is the difficulty in attributing therapeutic relevance to any observed biochemical finding. One powerful approach is to identify common biochemical targets, which are modified by drugs belonging to the same therapeutic class (e.g. mood-stabilizing agents) but possessing distinct chemical structures when administered in a "therapeutically relevant" paradigm. In this context, it is noteworthy that both valproate and lithium, with different chemical structures, belong to the same therapeutic class and cause considerable inhibition of protein kinase C (PKC). The PKC signaling pathway is clearly a target for the actions of two structurally highly dissimilar antimanic agents — lithium and VPA. Do these effects of lithium and VPA on PKC signaling have any clinical relevance? There is thus a clear need to investigate the potential efficacy of a direct PKC inhibitor in the treatment of acute mania. There is currently only one relatively selective PKC inhibitor available for human use- tamoxifen. Tamoxifen, a synthetic nonsteroidal antiestrogen, has been widely used in the treatment of breast cancer. Tamoxifen's potent inhibitory effects on PKC are striking. Recently, our group conducted an open-label study with tamoxifen and found a significant decrease in manic symptoms within 3-7 days. We undertook the present placebo-controlled study as a proof of the concept that PKC inhibition may have efficacy in the treatment of acute mania.
Methods: In a double-blind, randomized, placebo-controlled study, 16 patients between ages 18 and 60 years old with acute bipolar mania and a Young Mania Rating Scale (YMRS) ≥14 after a 2-7 day screening period were randomly assigned to receive tamoxifen (20-140 mg/day) (n = 8) or placebo (n = 8) for 2 weeks. Ratings were obtained on days 1-7 and 14. Primary efficacy was assessed by the YMRS.
Results: Baseline characteristics were comparable between the two groups. The mean age was 35.4 ± 7.8 years, there were 16 males/2 females, and 62.5% completed the study. Using all time points, the linear mixed model for YMRS showed a significant interaction between drug and time (F=2.76, df=7,67, p=.01). The tamoxifen group had significant change compared to baseline by day 5 of the study even after correcting for multiple comparisons. The difference between tamoxifen and placebo was significant after 2 weeks (tamoxifen from: 30.0 ± 6.7 to 11.7 ± 8.7; placebo: from 24.3 ± 6.7 to 23.0 ± 7.5). The effect size was very large for tamoxifen (d=1.76, 95% C.I.: 1.06 – 2.47), but near zero for placebo (d=0.13, 95% C.I.: -0.58 – 0.84). The tamoxifen group received an average daily dose of 110 mg ± 32. Response rates (>50% decrease in YMRS from baseline) was 4 of 8 (50%) on tamoxifen and 1 of 8 (13%) on placebo (Fisher's Exact Test, p=.08). Looking at the percent change from baseline to endpoint, tamoxifen (-41.1 ± 35.5) showed significantly greater improvement than placebo (5.0 ± 32.6) (t=2.70, df=14, p=.02). Symptoms of depression were not significantly worsened on tamoxifen versus placebo. Tamoxifen was overall well tolerated.
Discussion: Treatment with a PKC inhibitor led to rapid antimanic effects. To our knowledge, this is the first randomized, placebo-controlled monotherapy trial showing that the effects of lithium and valproate on PKC signaling have clinical relevance in terms of antimanic effects. Biochemical PKC signaling measures obtained during the study will be presented as well.

76. Cortical and Subcortical Gray Matter Volume Differences in Healthy Bipolar Offspring: Potential Neuroanatomical Risk and Protective Markers in Bipolar Disorder
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Sponsor: Mary L. Phillips

Background: A growing number of structural neuroimaging studies conducted in adults and adolescents have shown that Bipolar Disorder (BD) is associated with abnormalities of gray matter (GM) volume in cortical and subcortical regions known to support affect regulation. The goal of this study was to examine GM volume through out the brain in healthy bipolar offspring (HBO) relative to age-matched controls (CONT) as a way to identify possible neuroanatomical risk factors.
Methods: Participants between 7 and 18 years old were recruited with 16 having at least 1 parent diagnosed with BD and 16 serving as age-matched controls. All were free of Axis I diagnosis. High-resolution MRI structural images were acquired using a 3T Siemens scanner. We compared GM volume through out the brain using voxel-based morphometric (VBM) analyses using SPM5.
Results: Results showed that relative to the CONT group, the HBO group had significantly increased GM volume in the anterior insula, medial orbital frontal gyrus, and superior frontal gyrus. Differences remained for the medial orbital frontal gyrus once small volume corrections were applied. Correlational analyses revealed that these differences in GM volume were not related to age or puberty. Exploratory analyses examining gender effects revealed a significant group by gender interaction effect, indicating that girls in the HBO group had significantly increased GM volume compared to girls in the CONT group. There was no such difference observed between CONT and HBO boys. Results also showed decreased GM volume in parietal and middle prefrontal regions.
Discussion: Results of this study indicate that healthy bipolar offspring, girls in particular, have increased GM volume in prefrontal regions known to support affect regulation, namely the medial orbital frontal gyrus. These differences in cortical and subcortical GM volume in healthy bipolar offspring could be indicative of neuroanatomical risk markers for BD. Because these bipolar offspring are free of current psychopathology, these findings could also provide preliminary data for the identification of potential neuroprotective markers for BD. Prospective follow up research correlating these findings with longitudinal data on future psychopathology will allow us to test this hypothesis further.

77. Potential Predictive Factors for Development of Treatment-Emergent Suicidal Ideation in the Fluoxetine Placebo-Controlled Major Depressive Disorder Database
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Sponsor: David T. Wong

Background: A prior analysis of an open-label fluoxetine MDD study found female gender, younger age, baseline HAMD item 3 (HAMD-

3) score, treatment-emergent worsening of depression, more severe treatment-emergent psychomotor activation, to be statistically significant factors predicting the emergence of suicidal ideation (defined as a HAMD-3 score of 2-4). The objective of these present analyses was to evaluate potential factors predicting emergence of suicidal ideation in a large placebo-controlled, MDD fluoxetine database.
Methods: The database consisted of 16 studies. All analyses were stratified by study. Time to development of a HAMD-3 score ≥2 (from a baseline of 0 or 1) or to have a suicidal act was compared between treatments by Kaplan-Meier survival analysis. Baseline characteristics were tested individually using Cox proportional hazards model where the outcome was time to development of a HAMD-3 score ≥2 or to engage in a suicidal act regardless of baseline HAMD-3 score. Significant baseline characteristics were then included in a Cox model that also included treatment and time dependent covariates. The interactions of treatment with each time dependent covariate were also included in the model. If data were such that the maximum likelihood estimate of the hazard ratio (Cox model) could not be computed, the likelihood ratio test was used to compute a *P* value.
Results: Of 1292 fluoxetine- and 871 placebo-treated patients who began treatment with a HAMD-3 score of 0 or 1, or had a treatment-emergent suicidal act if the HAMD-3 score was ≥2, 0.8% of the fluoxetine- and 1.4% of the placebo-treated patients developed a HAMD-3 score of ≥3 or had a suicidal act (act: 0.19% fluoxetine, 0.20% placebo in all randomized patients); 7.4% of fluoxetine-treated patients and 10.6% of placebo-treated patients developed a HAMD-3 score ≥2 or had a suicidal act. Time to development of a HAMD-3 score ≥2 or to have a suicidal act was significantly longer with fluoxetine treatment ($P = .036$). Severity of depression and HAMD-3 score were significant baseline risk factors. In the full model, baseline severity of depression and HAMD-3 score were also significant. Treatment-emergent worsening of depression within both treatments and insomnia across both treatments were significant and the treatment interactions were not significant. Treatment-emergent major psychomotor activation within fluoxetine was significant. For placebo treatment and major psychomotor activation, outcome parameters could not be estimated by the maximization of the likelihood function. The likelihood ratio test found the hazard ratio for major psychomotor activation not significantly different from 1 within placebo treatment, but that a significant interaction between treatments existed, indicating a significant difference in hazard ratios for major activation between treatments
Discussion: For acute, treatment-emergent more severe suicidal ideation (HAMD-3 score ≥3) and acts, the incidence was so small that predictive factors could not be established in a total database of >2100 patients. When including less severe ideation (HAMD-3 score of ≥2) in the outcome, baseline severity of depression, baseline suicidal ideation, treatment-emergent worsening of depression, and treatment-emergent insomnia were statistically significant predictive factors common across both placebo and fluoxetine treatments in this database. More severe forms of psychomotor activation represented a statistically significant predictive factor for emergent suicidal ideation within the fluoxetine treatment group but not within the placebo group.

78. 8-Month Maintenance Treatment of Bipolar Depression (BD) with Lamotrigine (LAM) or Divalproex (DIV) plus LAM: Efficacy and Safety of Open-Label Combination of Divalproex ER (DIV) and Lamotrigine (LAM)

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Background: Bipolar disorder (BP) is chronic, characterized by relapses and recurrences with depressive symptomatolgy, in comparison to manic/hypomanic, accounting for the majority of time symptomatic (3 times greater in BP I and 37 times greater in BP II). Depressive episodes in BD, are more closely associated with treatment refractoriness, psychosocial impairment, and completed suicides than are either manic or hypomanic episodes, thus emphasizing the need to develop safe, efficacious and tolerable treatment regimens for this phase of the illness. Despite evidence supporting the efficacy of DIV and LAM in the maintenance treatment of BD, less than half of patients randomized to active drug treatment completed the monotherapy maintenance trials without developing a mood episode requiring intervention. When early discontinuation is factored as an indicator of overall effectiveness only one-third of the patients treated with either drug completed the trials without an early intervention. A large majority of patients with BP are on combination therapy, both acutely and long-term. Despite the frequent use of DIV+LAM in the maintenance treatment of BD, there is a lack of controlled data comparing the long term efficacy and safety of LAM+DIV to LAM monotherapy.
Methods: This randomized, double-blind, parallel group study (n=100, 50 in each group) aims to compare the safety and efficacy of LAM plus placebo vs DIV+LAM in the maintenance treatment of BD and includes an Open Phase (up to 8 weeks) and a Maintenance Phase (32 weeks). We present findings from the Open Phase of the study for the first 54 patients. BP I and II patients meeting criteria for BD at study entry or with an episode of BD within the past 6 months currently in resolution with treatment at study entry, as determined by DSM-IV/MINI criteria, were enrolled. During the Open Phase, all patients were treated with a combination of DIV and LAM, with a systematic addition of LAM to DIV as well as the addition of DIV to LAM. Other pharmacological agents in addition to DIV+LAM, with the exception of tricyclics antidepressants, MAO inhibitors, depot neuroleptics, ECT and the following atypical antidepressants (mirtazapine, trazadone and nefazadone), could be used to achieve improvement of depression. Patients were eligible for randomization to the Maintenance Phase after they met the following criteria for improvement for 2 consecutive weeks: 1) Montgomery- Asberg Depression Rating Scale (MADRS) total score ≤ 14, 2) Mania Rating Scale (MRS) score ≤ 14, and 3) Global Assessment Scale (GAS) score ≥ 51. Patients' whose depression was in resolution at study entry and met all randomization criteria were treated with a combination of DIV+LAM for at least 2 weeks to establish tolerability prior to being randomization.
Results: 32 (59%) patients met criteria for BD at study entry (mean MADRS score 25.7, range 15-41). Nineteen of the 32 (59.4%) depressed patients improved sufficiently to meet all criteria for randomization within 8 weeks. Three patients required the addition of an antidepressant to achieve randomization. Three (5.6%) patients who received at least 2 weeks of a combination of DIV+LAM developed a rash, all benign, that resolved with discontinuation of LAM.
Discussion: These preliminary findings demonstrate the safety of adding DIV to ongoing LAM treatment and vice versa, as well as the acute efficacy of the two in combination for patients with BD.

79. Lack of Association of AKT1 Gene and Bipolar Disorder in a North American Caucasian Cohort

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Sponsor: Arthur Rifkin

Background: Converging evidence have suggested that the AKT1 gene encoding a serine/threonine kinase may be involved in increased susceptibility to bipolar disorder. Linkage studies have reported positive LOD scores in the distal portion of chromosome 14q 22-32 in which the AKT1 gene is located. Second, the AKT1 is involved in lithium sensitive cell survival and proliferation pathway by modulating gene expression, protein synthesis, and cell cycle apparatus.

Methods: We recruited 206 caucasian participants for this study. Participants diagnosed with bipolar disorder (n=81; mean age 37.2 ± 12.3 years; female 46.9%) and study controls (n=125, mean age 36.9 ± 14.1 yrs, female 46.4%) were carefully characterized following an informed consent process. Diagnosis was established using the Structured Clinical Interview for DSM-IV. The AKT1 was genotyped across 11 single nucleotide polymorphisms (snps) namely rs2494730, rs2494732, rs2498799, rs2494734, rs2494735, rs3730358, rs10149779, rs11848805, rs2494747, rs1130214, and rs2494750. We used single nucleotide- and haplotype-based methods to examine the relationship of these sites on the AKT1 gene and bipolar disorder.
Results: We identified 3 major haplotype blocks within the AKT1 gene. However, no haplotype within the blocks was associated with bipolar disorder (all p-values >0.05). Similarly, none of the 11 snps were associated with bipolar disorder (all p-values >0.05) in this cohort.
Discussion: We found no association between the AKT1 gene and bipolar disorder in our cohort of Caucasian patients diagnosed with bipolar disorder.

80. Immunoreactivity for Calcium Channel Alpha2-Delta (α_2-δ) Type 1 Protein is Localized at Glutamate Synaptic Endings in Rat Brain and Neuronal Cell Cultures

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Background: Pregabalin and gabapentin are both specific and high-affinity ligands for the calcium channel alpha2-delta (α_2-δ) protein (1) that is an auxiliary protein subunit of voltage-gated calcium channels in skeletal muscle and brain (2). Recent structure-activity studies with a variety of compounds with varying affinity for binding to α_2-δ proteins have shown that high affinity binding is required for activity in animal models of anxiolytic action (3). There are four subtypes of α_2-δ protein, each coded by separate genes. Only α_2-δ subtypes 1 and 2 bind gabapentin and pregabalin with high affinity. Furthermore, mice with a genetic mutation targeted to a single amino acid of the calcium channel α_2-δ type 1 protein have greatly reduced binding of [^{3}H]pregabalin in forebrain (4), and also are insensitive to the anxiolytic-like action of pregabalin, but are still sensitive to GABA-ergic drugs (5). These findings suggest that α_2-δ type 1 is a novel anxiolytic drug target specific to gabapentin, pregabalin and closely related compounds.
Methods: Immunostaining used a specific monoclonal antibody (Sigma, anti-dihydropyridine receptor subunit α2, clone 20A) to α_2-δ type 1 with HRP-coupled secondary antibody for tissue sections; Alexa-fluor 594 conjugated secondary antibody for fixed neuronal cell cultures. PSD-95 protein was stained by a specific PSD-95 antibody (ABCAM) and Alexa-fluor 488 coupled secondary antibody. Rat tissue sections were obtained from Sprague-Dawley rats after fixation and paraffin imbedding (3 μm thick sections) followed by antigen retreival and standard immunohistochemistry. Cultures of neocortex cells were prepared on glass coverslips by standard methods, fixed with 4% formalin and processed for immunocytochemistry. Fluorescently stained cell cultures were observed by brightfield fluorescence microscopy with appropriate filters for independent imaging of Alexa 488 and Alexa 598 and also by DIC imaging with a 60x oil immersion objective. Images were obtained and overlaid using Metamorph imaging software.
Results: Previous work has not identified the localization of α_2-δ type 1 in subregions of brain, peripheral tissues or in neuronal cultures. We found very little α_2-δ staining of peripheral tissues such as spleen, kidney and lung (except for some staining in vascular smooth muscle) and relatively dense staining in skeletal muscle, cardiac atrial muscle, brain and spinal cord, in agreement with previous published studies of [[^{3}H]gabapentin and [^{3}H]pregabalin autoradiography (ref 4). Staining was particularly dense in several known glutamatergic synaptic pathways of brain, including many synaptic areas within the amygdala, the endings of the perforant path projection from entorhinal cortex to distal dentate granule cell dendrites of hippocampus and distal CA1 pyramidal dendrites of hippocampus and also in the granule cell-mossy fiber synapses onto CA3 hippocampal pyramidal neurons. Furthermore, extensive co-localization was seen in rat neocortical cell cultures between α_2-δ type 1 and the glutamate postsynaptic marker, PSD-95. Overall, there were high densities of α_2-δ staining in amygdala, layers 2-4 of entorhinal cortex, dorsal horn of spinal cord, and several other brain regions.
Discussion: We propose that these areas of dense staining are likely pharmacological sites of action for the anxiolytic-like actions of pregabalin in animal models and for its actions to prevent pain-related behaviors in animal models. REFERENCES: 1. Gee et al. (1996) J Biol Chem 271:5768-5776. 2. Arrikath & Campbell (2003) Curr Opin Neurobiol 13:298-307. 3. Belliotti et al. (2005)J Med Chem 48:2294-2307. 4. Bian et al. (2006) Brain Res 1075:68-80. 5. Lotarski et al. (2006) Soc Neurosci Abstr (in press).

81. Decreased Binding of [18F]MPPF in the Dorsal Raphe Nucleus (DRN) of Healthy Volunteers in Response to a Single Dose of Fluoxetine

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Sponsor: Claude de Montigny

Background: The present study was undertaken to determine whether brain imaging with PET may be used in human, to detect a biological response corresponding to the early change in extracellular 5-HT taking place at the onset of antidepressant treatment with an SSRI.
Methods: Healthy male volunteers (n=8; 19-35 year old) underwent two PET scans with [18F]MPPF (specific activity > 2Ci/μM), four to six weeks apart, following the randomized, double-blind administration of a single oral dose of placebo or fluoxetine (20 mg). A third PET scan was carried out while on placebo in 4/8 subjects to assess test-retest variability. The plasma levels of fluoxetine and norfluoxetine were measured by gas chromatography with electron-capture detection following derivatization with pentafluorobenzoyl chloride.
Results: Subjects did not report significant changes in mood after fluoxetine administration, with an average peak plasma level of fluoxetine of 21 ± 7 ng/ml during the PET scans. After fluoxetine, [18F]MPPF binding potential (BP) was reduced in the DRN of 7/8 subjects (37% ± 28; $p < 0.05$), while unchanged in all areas of 5 HT projection, including hippocampus. This far exceeded test-retest variability in DRN (4% ± 4). The availability of [18F]MPPF, estimated by the ratio of plasma to brain transport constant (R1) did not differ between placebo and fluoxetine conditions (1.02 ± 0.72 versus 1.08 ± 0.69).
Discussion: In the light of prior immuno-electron microscopic, beta-microprobe and PET studies in animals acutely treated with fluoxetine (Riad et al., 2004, *J Neurosci*, 4:5420-5426; Aznavour et al., 2006, *NeuroImage*, in press), such a regionally-selective decrease in [18F]MPPF binding is deemed consistent with internalization of 5-HT1A autoreceptors, with or without competition between endogenous 5 HT and the radioligand [18F]MPPF.

82. Anxiolytic and Analgesic Properties of Endocannabinoid Transport\FAAH Inhibitors

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Background: Phytocannabinoid agonists have been used for centuries to treat a number of mood and physiologic disorders including

anxiety, malaise, emesis, cachexia, inflammation, and muscle spasms associated with multiple sclerosis. Inconsistent dosing regimes, compound lipophilicity, abuse liability, and psychotropic side effects have limited the therapeutic utility of medical marijuana and synthetic derivates such as Nabilone and Marinol. We hypothesize that blocking endocannabinoid transport (ET) or fatty acid amide hydrolase (FAAH) using small molecule inhibitors will elevate endocannabinoid tone providing a more physiologic approach to cannabinoid receptor agonism and a better tolerated therapeutic outcome. Pharmacological tools were evaluated for their selectivity for these two currently known protein targets within the anandamide reuptake and catabolic pathway. A potent compound for anandamide reuptake inhibition was selected to test the hypothesis whether the motoric/sedative effects could be separated from the analgesic and anxiolytic efficacy in animal models of these behaviors.

Methods: Pharmacological evaluation of putative FAAH and endocannabinoid transport inhibitors was performed as previously described (Dickason-Chesterfield AK et al. Pharmacological Characterization of Endocannabinoid Transport and Fatty Acid Amide Hydrolase Inhibitors. Cell and Molecular Neurobiology.2007 DOI: 10.1007/s10571-006-9072-6). Behavioral testing was performed as previously described (Moore SA et al. Identification of a high-affinity binding site involved in the transport of endocannabinoids. 2005 Proc Natl Acad Sci USA, 102, 17852-17857).

Results: After testing many of the current compounds reported in the literature to be either selective or non-selective inhibitors of ET or FAAH, we found that all compounds evaluated blocked both targets with varying degrees of potency. A novel ET inhibitor was radiolabeled (3Hh-LY2183240) and was found to bind selectively to a plasma membrane-associated protein with high affinity in both cell lines and native rat brain tissue. Systemic administration of the ET inhibitor showed efficacy in animal models of analgesia and anxiety providing evidence of central penetration without inducing adverse motoric impairment.

Discussion: Separation of the psychotropic effects induced by cannabinoid agonists from their therapeutic benefit has been a long sought after goal in cannabinoid therapeutic development. Blockade of reuptake or catabolism has proven an effective mechanism to increase endogenous tone for biogenic amine neurotransmitters. The discovery of high potency reuptake blockers for anandamide transport has provided a method to test whether this hypothesis would generate behavioral responses in the absence of the motoric and sedative effects inherent in direct cannabinoid receptor agonists such as tetrahydrocannabinol. No motoric disruption was observed in rodents at concentrations of anandamide reuptake inhibition that generated analgesic and anxiolytic efficacy. These data indicate that blockade of endocannabinoid reuptake mechanisms may be potential therapeutic targets for anxiety and pain indications.

83. Macromolecular Abnormalities in Fronto-Striato-Limbic Regions and Remission of Geriatric Depression

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Sponsor: Jack D Barchas

Background: Fronto-striato-limbic abnormalities have been implicated in geriatric depression. To assess the relationship of fronto-striato-limbic integrity to the occurrence of remission of depression, we used magnetization transfer (MT) in older depressed patients undergoing treatment with escitalopram. MT imaging provides contrast based on the interaction of normally observed tissue water signal with protons bound by large macromolecules, including myelin. The magnetization transfer ratio (MTR) provides a measure of the difference in signal between free and macromolecule-bound water, and is viewed as a measure of myelin integrity. This study tested the hypothesis that depressed elders who remain symptomatic after escitalopram treatment have reduced MTR compared to those who achieve remission.

Methods: 32 subjects (age>60 years), with major depression (by SCID, DSM-IV) and a Hamilton Depression Rating Scale (HDRS) score of 20 or higher received escitalopram 10 mg for 8 weeks after a 2 week single-blind placebo phase. To determine the MTR, a 3D FLASH sequence was acquired both with and without an off-resonance magnetization pulse (TR = 31 ms, TE = 6 ms, 160x256 matrix (5/8 reduced), FOV=320 mm, 32 slices, 5 mm slice thickness, 0 mm skip). The identical sequence was repeated with an off-resonance pulse with a 1.5 kHz frequency offset. MTR was computed as the percent intensity difference between the on- and off-resonance pulse sequences. Images were normalized to Talairach space. Voxel-based analysis of MT data was conducted with a general linear model using age as the covariate. To control for type I error, a threshold was used that allowed only clusters of at least 100 voxels, all significant at $p < .05$. In addition, at least one of the voxels in the cluster had to be significant at $p < .005$.

Results: Of the 32 subjects (baseline after placebo lead-in HDRS mean: 23.4 , SD: 4.2), 16 achieved remission (final HDRS<=7) and 16 remained symptomatic. There were no significant differences between remitted and non-remitted subjects in age, baseline depression severity, cognitive impairment, or dosage of escitalopram. Relative to remitters, non-remitters demonstrated lower MTR in multiple fronto-striato-limbic regions, including white matter lateral to the left amygdala, left parahippocampal gyrus, and right inferior frontal gyrus, as well as bilateral subcallosal, insular, and lentiform regions. In addition, in non-remitters, lower MTR regions were observed in the left thalamus, splenium, precuneus, superior temporal, and inferior parietal regions along with bilateral midbrain and perirolandic regions.

Discussion: To our knowledge, this is the first report of lower MTR in fronto-striato-limbic regions of older patients with major depression who remained symptomatic compared to those who achieve remission. This observation is consistent with findings suggesting that fronto-striato-limbic abnormalities are associated with poor response to antidepressants. Diffusion tensor imaging studies suggest that reduced fractional anisotropy in white matter lateral to the anterior cingulate predicts poor remission of geriatric depression to citalopram. The metabolism, activity, and processing of some fronto-striato-limbic regions may predict antidepressant response. Similarly, executive dysfunction has been associated with fronto-striato-limbic abnormalities and predicts limited response of geriatric depression to citalopram. Limitations of this study include the lack of a placebo arm and limited neuropsychological assessment.

84. Corticotropin Releasing Factor (CRF) mRNA is Decreased in the Hypothalamic Paraventricular Nucleus (PVN) of Macaques Treated with Ovarian Steroids

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Sponsor: Daniel Dorsa

Background: CRF in the PVN is elevated by stress and it has been implicated in the etiology of depression. Rodent CRF neurons in the PVN express serotonin receptors and nuclear estrogen receptor beta (ERβ) receptors. We have shown that ovarian steroids act in macaque serotonin neurons to alter gene and protein expression in a fashion that suggests increased serotonin neurotransmission would result. We now question how ovarian steroid replacement, and presumably elevated serotonin, converge to affect the expression of CRF in the PVN of surgically menopausal macaques. Recently (Society for Neuroscience, 2006) we reported that immunodetectable CRF was significantly decreased by hormone replacement across 5 levels of the PVN.

In this study, we tested the hypothesis that CRF mRNA would be decreased by ovarian hormone replacement in a manner similar to CRF protein.
Methods: Rhesus monkeys were spayed and treated for 28 days with placebo (controls), estrogen (E), progesterone (P) or E+P (14 days E+ 14 days E and P) using Silastic capsules implanted in the periscapular region (n=5/treatment). A CRF cDNA was prepared with total RNA from monkey hypothalamus, RT-PCR and primers directed to the region of monkey CRF spanning nucleotides 688-889. Perfusion-fixed sections (25 μm) at 5 levels of the PVN were hybridized to the CRF riboprobe and washed under stringent conditions. The sections were apposed to x-ray film, which was then developed. The CRF signal was analyzed with NIH Image and subjected to statistical analysis with Instat.
Results: CRF mRNA signal, as expressed by optical density and pixel area, was significantly suppressed by ovarian hormone replacement at levels 1, 2 and 3 of the PVN. CRF signal was nearly significantly suppressed at levels 4 and 5. The mean optical density of all levels equaled 32.4±8.8, 12.4±2.2, 8.3±1.8 and 13.3±3.6 in the control, E, P and E+P treated groups, respectively (ANOVA, $p < 0.015$). The mean pixel area of all levels equaled 900±280, 258±71, 160±61, and 354±80 in the control, E, P and E+P treated groups, respectively (ANOVA, $p < 0.015$). Posthoc pairwise comparison indicated that the E, P and E+P treated groups were significantly less than the spayed control group, but the treated groups were not different from each other.
Discussion: CRF governs the hypothalamic-pituitary-adrenal axis which is frequently hyperactive in major depression and CRF is elevated in many models of stress. Thus, CRF is perceived as a pivotal neuropeptide in affective regulation. Prior studies with rodents suggested that E elevates CRF in the PVN. However, we find that E, P and E+P treatment, simulating a menstrual cycle in primates, decreases CRF mRNA and protein in the PVN. This effect could be due to elevated serotonin acting through serotonin receptors on CRF neurons, or due to ovarian steroids acting through steroid receptors in CRF neurons, or both. The decrease in CRF with ovarian steroid replacement in primates suggests that ovarian function may facilitate stess resilence in normal individuals throughout the reproductive years.

85. Quetiapine Addition to Serotonin Reuptake Inhibitors in Non-Refractory Obsessive Compulsive Disorder

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Background: Risperidone, olanzapine, and quetiapine have shown to be effective as add-on to serotonin reuptake inhibitors (SRIs) for treatment refractory patients with obsessive-compulsive disorder (OCD). Up till now, it is unknown whether antipsychotic addition to SRIs may benefit treatment-naive or treatment-free patients with OCD.The objective of the present study was to determine whether addition of quetiapine to SRIs is superior to SRI treatment alone for non-refractory OCD patients.
Methods: Eighty-two patients with primary OCD according to DSM-IV criteria, were randomly assigned in a 10-week, double-blind trial to receive dosages titrated upward to 300 mg/day of quetiapine to citalopram 60 mg/day, or 60 mg/day of citalopram. None of the patients had been treated with an SRI at maximum dose during at least 12 weeks. Primary efficacy was assessed by the change from baseline on the Yale-Brown obsessive-compulsive scale (Y-BOCS), and response was defined as more than 35 % reduction on the Y-BOCS. Depression was rated with the 17-item Hamilton Rating scale for Depression (HAM-D) and anxiety was evaluated with the Hamilton Anxiety Scale (HAM-A).
Results: As measured by the reduction in Y-BOCS scores following an ITT-LOCF analysis, quetiapine addition (12.2 ± 6.1) was superior to placebo (8.6 ± 6.4) ($F=5.9$, $P=0.02$). Equally, on the CGI, quetiapine addition was superior to placebo with a mean CGI-improvement score of 2.2 ± 1.2 versus 1.5 ± 1.2 ($F=4.4$, $P=0.04$). Twenty-four patients (70%) responded in the quetiapine addition group versus 20 (50%) in the placebo group using the 35% Y-BOCS response criterion. The HAM-A and HAM-D did not differ significantly between the two treatment groups. Six patients dropped out in the quetiapine + citalopram group versus 3 in the citalopram + placebo group.
Discussion: The results of this study suggest that addition with quetiapine to SRIs might improve treatment outcome in non-refractory OCD patients.

86. Proton Magnetic Resonance Spectroscopy in Children and Adolescents with Severe Mood Dysregulation

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Background: Irritability is common in youth presenting for psychiatric evaluation. It is a DSM-IV diagnostic criterion for bipolar disorder (BD), major depressive disorder (MDD), and anxiety disorders, and it is seen in numerous other child psychiatric diagnoses, including oppositional defiant disorder, intermittent explosive disorder (IED), and attention deficit hyperactivity disorder (ADHD). Although irritability is a ubiquitous symptom, little is known about its underlying neurobiology, hampering the development of targeted treatments. Prior magnetic resonance spectroscopy (MRS) studies in youth with BD, MDD, and ADHD, who often demonstrate irritability, have found neurochemical abnormalities in (a) glutamate/glutamine (GLX), a combined marker of the major excitatory neurotransmitter, glutamate, that can not be fully resolved at low strength MR fields, (b) inositol (Ins), whose aberrantly increased concentration during mania is reduced by lithium, and (c) N-acetyl aspartate (NAA), a putative marker of mature neurons that is increased by lithium. However, these studies have been limited by small sample size and/or ongoing psychotropic medication use. We tested the hypothesis that these metabolites would be increased in a large sample of unmedicated youth with severe, chronic, impairing irritability, compared to healthy controls.
Methods: 1.5 Tesla 1H MRS was conducted in 48 controls and 36 youth with severe mood dysregulation (SMD; see Leibenluft et al. 2003: chronic irritability, hyperarousal, and developmentally inappropriate reactivity to negative emotional stimuli with functional impairment). LC Model was used to quantify neurometabolites normalized to creatine (Cr), in 8 cm3 voxels placed in the orbitofrontal cortex (right), occipital (precuneus, central), parietal (left) and temporal regions (hippocampus, left).
Results: Compared to controls, SMD subjects had significantly higher GLX/Cr in the occipital region ($p=0.04$) and lower Ins/Cr in the temporal lobe ($p=0.05$). There were no other significant between-group differences. SMD youth with ADHD and no mood or anxiety disorders (N=14) had significantly increased frontal GLX/Cr compared to controls ($p=0.04$).
Discussion: Unlike prior studies of BD, we did not find frontal MRS abnormalities, and we found decreased, rather than increased, inositol. Since inositol depletion may mediate lithium's anti-manic effect, our findings suggest that lithium may not effectively treat children with chronic irritability. Additional work could determine if there is an association between increased GLX in the precuneus and social dysfunction in SMD youth. Lastly, our results in SMD youth with only ADHD are consistent with prior findings of increased frontal GLX/Cr in ADHD youth.

87. Serotonin Transporter Binding in Major Depressive Disorder and Bipolar Disorder Assessed using [11C]DASB and Positron Emission Tomography

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Sponsor: Peter Schmidt

Background: Abnormalities of the serotonergic system play a role in the pathophysiology of depressive disorders based upon based on evidence from neuroimaging, post-mortem, and genetic studies. However, to date 5-HTT levels have not been compared directly between depressed subjects with major depressive disorder (MDD) and bipolar disorder (BD). Thus, the present study included unmedicated depressed MDD and BD subjects and healthy-controls who underwent [C-11]DASB PET which afforded higher sensitivity and specificity for the 5-HTT than previously available 5-HTT-radioligands.
Methods: Currently-depressed, unmedicated subjects meeting DSM-IV criteria for MDD (n=18, 13-female; mean age±sd=35±9) or BD (n=18, 12-female, 30±9), and 34 healthy-controls (25-female; 35±8) underwent [C-11]DASB PET scanning. The main outcome parameter was 5-HTT binding potential (BP), derived using a modified reference-tissue model (Ichise et al. J Cereb Blood Flow Metab 2003, V23:1096-1112) using cerebellum as the reference region.
Results: Mean 5-HTT BP differed significantly across the three samples using MANOVA and these were accounted for by increases in MDD subjects relative to controls in the thalamus (27%, pcorr<0.0001), insula (15%, pcorr=0.020), striatum (12%, pcorr=0.040), and periaqueductal gray (PAG, 22%, pcorr=0.009) and a trend in the same direction in pgACC (16%, pcorr=0.061 or puncorr=0.036), and by increases in BD subjects relative to controls in the DCC (21%, pcorr=0.031) and a trend in the same direction in thalamus (14%, pcorr=0.063), and finally, by increases in the MDD relative to the BD sample in PAG (22%, pcorr=0.025), after Bonferroni correction for comparisons across nine regions. Mean 5-HTT BP in midbrain raphe (F=1.30, p=0.278), sgACC (F=2.25, p=0.114, and PCC (F=2.35, p=0.103) did not differ in MDD or BD subjects relative to controls. Post-hoc voxel-by-voxel analysis confirmed these increases in MDD and BD-subjects versus controls in thalamus, insula and caudate and in PAG, hippocampus and hypothalamus in MDD-subjects and a mPFC region in BD-subjects. Lower binding in the BD-subjects versus the controls was detected in the brainstem at the level of the pontine raphe (T=3.25). Mean 5-HTT BP in the thalamus correlated negatively with the severity of depression (r=-0.517, p=0.034, n=17), and duration of illness (r=-0.580, p=0.012, n=18), during the depressed phase of MDD. In addition, 5-HTT BP in the PAG correlated positively with the severity of anxiety (r=0.545, p=0.024, n=17).
Discussion: Serotonin transporter density and/or affinity were increased in the thalamus and insular cortices during the depressed phase of both MDD and BD. These findings are consistent with previous studies showing increased 5-HTT binding in the thalamus (Ichimiya et al. Biol Psychiatry 2002, V51:715-722), and cingulate cortices (Reivich et al. J Affect Disord 2004, V82:321-327) using [11C](+)McN5652 and in the thalamus, PFC, pgACC and striatum in MDD-subjects manifesting negativistic attitudes versus controls (Meyer et al. Arch Gen Psychiatry 2004, V61:1271-1279). Increased 5-HTT binding in the mPFC is compatible with elevated 5-HTT RNA transcripts reported in the frontal cortex of individuals with BD post-mortem (Sun et al. Br J Psychiatry Suppl 2001, V41:s137-141). PAG 5-HTT BP was elevated in MDD-subjects relative to both controls and BD-subjects. This is the first study to report significant differences in 5-HTT binding between unmedicated BD and MDD-subjects.

88. The Influence of Negative Affect on Working Memory in Bipolar-I Disorder Patients and Healthy Volunteers: An fMRI Investigation

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Background: Cognitive impairment is a prominent feature of depressive episodes in both major depression and bipolar disorders. It contributes to impairments in psychosocial functioning and is suspected to adversely impact response to cognitive behavior therapy (CBT). Little is known about the functional neuroanatomy that underlies concentration and memory impairments associated with depressed mood. The purpose of the present study was to investigate the impact of negative affect on working memory in depressed patients with bipolar-I disorder and healthy volunteers.
Methods: Twenty-four healthy control participants and 12 depressed individuals with bipolar-I disorder (BP-I) completed an emotional 2-back working memory paradigm in conjunction with functional magnetic resonance imaging (fMRI) at 3T. Briefly, participants were serially shown individual letters on a computer screen and were instructed to respond by botton press as to whether or not a given letter was identical to the letter presented two letters earlier (i.e. L, M, L = yes; L, M, P = no). Letters were presented superimposed on pictures of neutral, negative or positive affective valence taken from the International Affective Picture System (IAPS). We hypothesized that concurrent processing of negative affect while engaging in the working memory task would result in increased activation in emotion relevant areas (e.g., amygdala) and tax cognitive systems involved in manipulation and updating of information, performance monitoring, and error control (anterior cingulate [ACC] and dorsolateral prefrontal cortex [DLPFC]) in both groups, but moreso in the bipolar-I group.
Results: Consistent with our hypotheses, control participants showed increased amygdala activation associated with negative (vs. neutral) affect. They also exhibited increased activation in the ACC and DLPFC in the negative affect (vs. neutral) condition. Results of the BP-I group will also be reported.
Discussion: These findings have relevance for proposed future clinical trials assessing predictors and mediators of response to CBT in BP-I using fMRI.

89. Molecular, Pharmacological and Behavioral Characterization of LY451395: A Positive Allosteric Modulator of Glutamate AMPA Receptors

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Background: Accumulating evidence suggests that dysfunction of glutamatergic signaling in the hippocampus and prefrontal cortex (PFC) may contribute a variety of psychiatric disorders, including cognitive deficits and depression. One therapeutic strategy designed to enhance glutamatergic signaling involves positive allosteric modulation of AMPA receptors which mediate rapid excitatory transmission in the CNS. The principal action of these modulators is to enhance AMPA receptor signaling by attenuating the deactivation and/or desensitization processes of the receptor/channel complex. In addition, positive modulators can have secondary consequences including, recruitment of voltage-dependent NMDA receptor synaptic transmission, as well as facilitation of long-term potentiation (LTP), a possible cellular substrate for memory encoding. Positive modulators also can augment the expression of brain-derived neurotrophic factor (BDNF) which itself can restore LTP in middle-aged animals and also may be a mediator and/or marker of antidepressant activity. Collectively, these data have prompted preclinical and clinical studies on the therapeutic utility of AMPA receptor modulators in a variety of psychiatric disorders.

Methods: The present studies investigated the molecular, pharmacological and behavioral properties of LY451395, a prototype from a novel class of biarylsulfonamide compounds.
Results: Crystallographic analyses reveal that a single molecule of LY451395 binds at the dimer interface of adjacent AMPA receptor ligand binding cores and is predicted to act by stabilizing the open conformation of the channel. This 1:2 stoichiometry confers high potency in functional assays using recombinant receptors (EC50=69 nM) or native receptors expressed on PFC (EC50=370 nM) and hippocampal (EC50=380 nM) neurons. Modulation by LY451395 is evident only in the presence of agonist, confirming its allosteric mode of action, involves suppressing receptor desensitization consistent with crystallographic predictions, and is blocked by selective antagonists. LY451395 also is capable of enhancing AMPA-induced expression of BDNF in primary cultures of cortical and hippocampal neurons which depends in part on calcium influx through L-type calcium channels. In vitro autoradiography studies demonstrate that [3H]LY451395 binds with high affinity (Kd=9.7 nM) to native receptors and displays high levels of binding in the prefrontal cortex and hippocampus. Consistent with these findings, systemic administration of LY451395 increases glucose utilization in these brain regions. In electrophysiological studies in vivo, LY451395 (0.1-100 μg/kg, i.v.) directly enhances the excitatory effects of iontophoretically applied AMPA and indirectly potentiates the effects of NMDA. As well, LY451395 augments the discharge of PFC neurons evoked by stimulation of hippocampal glutamatergic afferents, an effect that can be blocked by selective AMPA receptor antagonists. The pronounced effects of LY451395 on glutamate transmission and BDNF expression suggested that it may be efficacious in animal models assessing cognitive function and antidepressant-like activity. Indeed, LY451395 reduces total errors made by rats in the 8-arm radial maze (0.1-1.0 mg/kg, p.o.) and the Morris water maze (0.001-0.1 mg/kg, p.o.). LY451395 also reduces immobility times in the forced swim test in rats (1.25-15 μg/kg, p.o.) and mice (2.5-100 μg/kg, p.o.) to levels equivalent to those of imipramine (15 mg/kg, i.p.).
Discussion: Together, these data demonstrate that LY451395 is a potent, selective and centrally-penetrant positive allosteric modulator of AMPA receptors that has activity in animal models predictive of therapeutic impact upon cognition and mood.

90. Effects of Initial Dosing Strategies of Duloxetine on Tolerability and Efficacy in Patients with Major Depressive Disorder

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Background: Patients often discontinue antidepressant treatment due to early side effects. Starting at a lower dose or taking the medication with food is often recommended to mitigate initial adverse events, but these strategies have not been well studied. Duloxetine is a serotonin-norepinephrine reuptake inhibitor with a recommended dose of 60mg QD. A previous open-label duloxetine study suggested that starting duloxetine at 30mg QD for one week followed by escalation to 60mg QD may lessen the risk of initial nausea with only a short-lived impact on efficacy. This study compared starting doses of duloxetine taken with or without food.
Methods: This double-blind, parallel design trial was conducted in adult outpatients with major depressive disorder(MDD). Patients were randomized in a 3 x 2 complete factorial arrangement to one of three starting dose groups: 30mg QD (n=219), 30mg BID (n=213), or 60mg QD (n=215) and to one of two food groups: by instruction to take study drug with food or not within one hour of eating. After 1 week on the starting dose, all patients were dosed at 60mg QD for the remaining five weeks of treatment. The primary objective of the study was to compare the rate of treatment emergent nausea in the 30mg QD group versus the 60mg QD group based on item 112 (nausea) of the Association for Methodology and Documentation in Psychiatry adverse event scale (AMDP-5). A key secondary objective was to evaluate mean changes on AMDP-5 item 112 using an analysis that included dose group, food group, and their interaction. Other secondary objectives included mean changes on an a priori determined common adverse events list and discontinuations due to adverse events. Efficacy was primarily evaluated by the 17-item Hamilton Depression Rating Scale(HAMD).
Results: No significant differences were found between starting doses of 30mg QD and 60mg QD on the primary analysis of rate of treatment-emergent nausea. However, on the secondary mean change analysis, both the main effect of food group and the starting dose group by food group interaction were significant at Week 1. These results differed from the primary analysis because the mean change analysis assessed changes in both rate and severity of nausea. Further, there was a main effect of food. Taking duloxetine with food improved initial nausea—with the greatest benefit of food among those patients who started at 60mg QD. When taking duloxetine without food, patients starting at 30mg QD had improved nausea compared with those at 60mg QD. In patients taking duloxetine without food discontinuation rates due to adverse events were 3.6%, 14.0%, 10.2% versus those taking it with food at 5.4%, 7.5%, and 7.4% for 30mg QD, 30mg BID, and 60mg QD respectively.All starting dose groups showed significant baseline to endpoint improvements, as measured by mean changes in the HAMD. However, patients initially dosed at 60mg QD showed significantly greater improvement at Week 2 than those initially dosed at 30mg QD or 30mg BID. For the remaining 4 weeks of treatment, mean change did not differ among initial dose groups.
Discussion: Taking duloxetine with food or starting at 30mg QD appeared to improve initial tolerability. Starting 30mg BID did not improve tolerability compared to 60mg QD. The starting dose of 30mg QD in the first week produced a transient disadvantage in efficacy compared to a starting dose of 60mg QD.

91. Lithium Monotherapy Versus the Combination of Lithium and Divalproex for Rapid Cycling Bipolar Disorder Comorbid with Substance Abuse or Dependence: A 6-Month, Double-Blind, Maintenance Trial

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Sponsor: Joseph R. Calabrese

Background: There is currently no FDA approved treatment for bipolar disorder (BD) comorbid with substance use disorders (SUDs) despite evidence that over half of bipolar patients suffer from alcohol or drug abuse/dependence (Regier et al., 1990). A recent trial has shown that the addition of divalproex (DVX) to Lithium (Li) was superior to placebo at decreasing heavy drinking in patients with bipolar I disorder and comorbid alcohol dependence (Salloum et al., 2005). However, the effectiveness of these agents in preventing mood episode recurrence in patients with BD complicated by SUDs is unclear. The present study is believed to be the first prospective, maintenance trial to compare the combination of Li and DVX to Li monotherapy at preventing recurrence in patients with BD and comorbid SUDs.
Methods: Outpatients with rapid-cycling bipolar I or II disorder who experienced a manic, hypomanic, or mixed episode within 3 months of study entry and who met DSM-IV criteria for substance abuse or dependence within 6 months prior to screening were eligible for enrollment. Participants were initially treated with the open-label combination of Li and DVX for up to 6 months. The study employed a moderately enriched design, thus only subjects who stabilized (HAM-D17 $\leq$ 20, YMRS $\leq$ 12.5, GAS $\geq$ 51, blood levels of Li $\geq$ 0.8

meq/L and DVX ≥ 50 ug/ml) on the treatment combination for ≥ 4 weeks were randomized (stratified for bipolar type I or II) to double-blind therapy with Li monotherapy or to continue on the combination of Li and DVX.
Results: A total of 148 subjects received open-label treatment with Li and DVX. The majority of subjects were male (64%) and had bipolar I disorder (75%). One-fifth of subjects (N=30) met stabilization criteria. Lack of adherence and adverse events accounted for 42% and 10% of dropouts, respectively. The combination of Li and DVX was ineffective at improving mood symptoms for 26% of subjects, with equal numbers experiencing refractory depression (N=19) or refractory manic/hypomanic/mixed states (N=19). Thirty subjects achieved stabilization with combination treatment and were randomized to continue on Li (N=16) or Li and DVX (N=14). Of the subjects who stabilized, 63% (N=19) were using alcohol and 70% (N=21) were using cannabis or cocaine at study entry. Combination treatment led to remission of the alcohol use disorder in 58% (N=11) and the cannabis or cocaine use disorder in 36% (N=4). Survival time in the study until discontinuation for any reason was not different between Li and the combination of Li and DVX (p=0.617). The median time to discontinuation for any reason was 10.4 weeks for Li and 10.0 weeks for the combination of Li and DVX. Subjects were statistically more likely to relapse into manic/hypomanic/mixed states (N=13) than into depression (N=4; p=0.029).
Discussion: Unlike previous reports that show refractory depression to be more prevalent in rapid cycling BD uncomplicated by SUDs, the results of this trial show that bipolar patients with comorbid SUDs suffer equally from refractory depressive or manic/hypomanic/mixed symptoms. Relapse into manic/hypomanic/mixed states during maintenance treatment was significantly more common than relapse into depression. Rigorous stabilization criteria may have contributed to the high rate of attrition during the open-label stabilization phase but is consistent with other maintenance trials employing an enriched design. If effective, the combination of lithium and divalproex does not appear to have a large or moderately large effect size when employed for the treatment of patients with rapid-cycling BD and comorbid SUDs. Supported by a Supplement to R01 MH-50165. Medication provided by Abbott and GlaxoSmithKline.

92. Switching to Duloxetine from Other Antidepressants: A Regional Multicenter Trial Comparing Two Switching Techniques

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Background: Patients with major depressive disorder (MDD) are commonly switched to a different antidepressant in the event of suboptimal treatment response, but there are few published data to guide clinicians on the best way to switch. The objective of this study was to compare 2 methods of switching selective serotonin reuptake inhibitor (SSRI) non- or partial responders to duloxetine.
Methods: Study participants were outpatients of at least 18 years of age who met DSM-IV criteria for MDD despite having taken an SSRI antidepressant for at least 6 weeks, and who had a Hamilton Depression Rating Scale ($HAMD_{17}$) total score of ≥15 and a Clinical Global Impression of Severity (CGI-S) score of ≥3. Patients were randomized to either abrupt discontinuation of SSRI and simultaneous initiation of duloxetine (direct switch; DS) or tapered discontinuation of an SSRI over 2 weeks and simultaneous administration of duloxetine (start-taper switch; STS). The primary outcome was to demonstrate the non-inferiority of DS compared with STS, as measured by a comparison of mean change from baseline to endpoint in the $HAMD_{17}$ total score in the 2 switch groups after 10 weeks of duloxetine (60-120 mg/day) treatment. Additional efficacy measures included response rates (≥50% decrease in $HAMD_{17}$ total score), remission rates ($HAMD_{17}$ total score ≤7 at endpoint), and visual analogue scales for pain (VAS). Safety and tolerability were assessed via reporting of adverse events (AEs), vital signs, and laboratory analytes.
Results: A total of 368 SSRI (predominantly paroxetine, citalopram, fluoxetine, sertraline, or escitalopram) non- or partial responders were randomized to DS or STS. A high proportion of patients completed the 10 weeks of duloxetine treatment in both switch groups (84.2% in the DS group vs 86.5% in the STS group; $P = .558$). There was a significant improvement in depressive symptom severity in both switch groups as measured by mean change from baseline to endpoint in $HAMD_{17}$ total score, but no difference between the groups (-10.23 DS vs -10.49 STS; $P = .698$), and the criterion for non-inferiority of the DS group vs the STS group was met. The switch groups also were similar with respect to response rates (54.4% DS vs 59.6% STS; $P = .360$), remission rates (35.7% DS vs 37.2% STS; $P = .849$), and other secondary efficacy measures. There was a significant within-group improvement for all efficacy measures ($P < .01$) including VAS for pain. Subgroup analysis according to SSRI at study entry did not suggest superiority of one switch group over the other for any of the individual SSRIs, nor differential efficacy following duloxetine switch. Few patients experienced a serious adverse event (5 patients during the 10 weeks of treatment and 1 during the taper period), and there was a low rate of discontinuations due to AEs (6.6% DS vs 3.8% STS). Headache (13.1% DS vs 9.7% STS), dry mouth (10.4% DS vs 11.9% STS), and nausea (8.2% DS vs 8.1% STS) were the most frequently reported AEs, and no AE was reported by significantly more patients in one group compared with the other. An excess of AEs was not evident following switch from any particular SSRI.
Discussion: Efficacy, safety, and tolerability outcomes following direct switch from SSRI to duloxetine were similar to those observed for start-taper switch. Overall, switch to duloxetine was associated with significant improvements in both emotional and painful physical symptoms of depression, and was well tolerated and safe, regardless of which SSRI the patient was taking at study entry.

93. A Preliminary Metabolomic Analysis of Older Adults with and Without Depression

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Sponsor: Redford Williams

Background: Metabolomics, the global science of biochemistry, is an emerging field that enables detection and quantification of small molecules involved in metabolic and signaling pathways. Metabolic signatures for disease and its treatment could provide valuable biomarkers and insights about disease mechanisms. In this pilot study, we evaluate the potential of metabolomics in the study of older depressed patients.
Methods: All subjects were enrolled in the Conte Center for the Neuroscience of Depression in Late Life at Duke University Medical Center. We performed a metabolomic analysis of blood plasma from nine depressed, eleven remitted, and ten never-depressed older adults. Approximately 800 metabolites were analyzed, with comparisons made among the three groups. All subjects provided written, informed consent for the study.
Results: Metabolites that were altered in currently depressed patients when compared with controls included several fatty acids, glycerol and gamma-aminobutyric acid (GABA). Analyses comparing concentrations in remitted and currently depressed patients revealed a pattern of metabolite alterations similar to the control versus currently depressed analyses. One difference observed in the remitted patients relative to the depressed patients was elevation of the concentration of the ketone 3-hydroxybutanoic acid.
Discussion: These observations suggest that the depressed state may be associated with alterations in the metabolism of lipids and neurotransmitters, and that treatment with antidepressants adjusts some of the aberrant pathways in disease so that the patients in remission have a metabolic profile more similar to controls than to the de-

pressed population. These results will need to be examined and validated in larger longitudinal cohorts.

94. Fibromyalgia: Preliminary Evidence for Sex Specific Prenatal Programming of Stress Related Disorders

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Background: Fibromyalgia (FM) is considered a stress related disorder, which is associated with a disturbed pituitary adrenal axis. FM is predominantly present in women.
Methods: Two cohorts of patients diagnosed with fibromyalgia were studied. The first cohort (N=103) was explored with a new diagnostic tool (Neuropattern), which attempts to assess stress related changes of discrete neuroendophenotypes. The second cohort underwent a laboratory stress test (TSST).
Results: When compared to healthy controls, FM patients report significantly more prenatal load during the pregnancy of their mothers, such as loss of the partner, trauma, low social support, and a shorter gestational length. We further observed a blunted rise of salivary cortisol after awakening in FM patients. However, blunted cortisol levels were only observed in FM patients with short gestational length. Since we have previously shown that the cortisol rise after awakening correlates with the cortisol release after Synacthen challenge, these findings suggest low adrenal capacity only in those FM patients which report evidence for prenatal load. In the TSST, we observed a tendency towards higher ACTH- but lower cortisol responses to psychosocial stress.
Discussion: Data from human and animal research suggest that a stress induced increase of cortisol in the pregnant mother may programm low capacity of the adrenals of the female offsprings. Recent data from this laboratory provide evidence that prenatal load results in an enhanced ACTH- but blunted cortisol response to psychological stress only in women with a history of prenatal stress. Thus, we conclude that prenatal stress of the mother may program low adrenal capacity in the female fetus, and that low adrenal responsivity to intense stress in later life may put these subjects at risk for developing fibromyalgia. We currently explore if FM patients who were unable to sufficiently buffer a stress response developed a pain- and fatigue memory.

95. Selegiline Transdermal System for the Treatment of Major Depressive Disorder: A Symptom-Based Analysis of Placebo-Controlled Efficacy Trials

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Background: Selegiline transdermal system (STS), the first dermal-delivered MAOI antidepressant, was recently approved by the Food and Drug Administration for treatment of major depressive disorder (MDD). Placebo-controlled trials of STS show both short-term efficacy (6 mg/24 hr to 12 mg/24 hr) [1-3] and prevention of relapse (6 mg/24 hr).[4] In deciding on specific pharmacotherapy for MDD, clinicians are often influenced by presenting symptoms.[5] The objective of this analysis was to investigate treatment effects of STS for specific symptoms of MDD, based on a line-item analysis of the 28-item Hamilton Rating Scale for Depression (HAM-D28) and the Montgomery-Asberg Depression Rating Scale (MADRS).
Methods: Data from 5 short-term, randomized, placebo-controlled efficacy trials in patients meeting DSM-IV criteria for MDD were utilized for this post-hoc analysis. Separate HAM-D28 and MADRS line-item analyses were carried out for 2 trials meeting *a priori* primary efficacy endpoints, as well as for all the efficacy trials combined. Confidence intervals (95%) were computed for treatment differences. Cohen's *d* effect sizes were calculated to compare differences between STS and placebo.
Results: Using 2 pooling strategies, STS showed significant ($p<0.05$) positive treatment effects on core symptoms of depression (HAM-D Bech-6 subscale: item 1-depressed mood; 2-guilt; 7-work and activities; 8-retardation; 10-psychic anxiety; 13-general somatic symptoms), anergic symptoms, hypersomnia and increased appetite (reverse vegetative symptoms), suicide, and genital symptoms (libido). STS treatment was associated with significant improvement on 8 of the 10 MADRS items with the exception of reduced sleep and appetite. STS treatment effects for MADRS symptom items were most prominent for poor concentration, lassitude, and sadness. These findings indicate that STS has a therapeutic profile similar to that described for other MAOI antidepressants.
Discussion: STS, an MAOI antidepressant that potentiates the activity of 3 key neurotransmitters (serotonin, norepinephrine, and dopamine), appears efficacious for a spectrum of individual depressive symptoms rated by the HAM-D28 and MADRS, including core depressive symptoms, anergic and reverse vegetative symptoms, and anxiety. Item analyses can provide additional guidance to clinicians in individualizing drug therapy. References 1. Bodkin JA, Amsterdam JD. Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. Am J Psychiatry 2003;64:208-214 2. Amsterdam JD. A double-blind, placebo-controlled trial of the safety and efficacy of selegiline transdermal system without dietary restrictions in patients with major depressive disorder. J Clin Psychiatry 2003;64:208-214 3. Feiger AD, et al. Selegiline transdermal system for the treatment of major depressive disorder: am 8-week, double-blind, placebo-controlled, flexible-dose titration study. J Clin Psychiatry 2006; in press 4. Amsterdam JD, Bodkin JA. Selegiline transdermal system (STS) in the prevention of relapse of major depressive disorder: a 52-week, double-blind, placebo-substitution, parallel-group clinical trial. J Clin Psychopharmacol 2006; in press 5. Zimmerman M, et al. Which factors influence psychiatrists' selection of antidepressants? Am J Psychiatry 2004;161:1285-1289

96. MRS Studies of Glutamate and GABA in Bipolar Disorder

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Background: Bipolar disorder (BD) is associated with abnormalities in cellular metabolism and synaptic neurotransmission. The synthesis and breakdown of the neurotransmitters glutamate (Glu) and gamma-aminobutyric acid (GABA) are dependent on these processes. Thus, their levels in vivo are indirect measures of cellular and synaptic activity. We set out to measure Glu and GABA levels in BD subjects and healthy controls using proton magnetic resonance spectroscopy (MRS). We chose to study BD patients in an acute manic or mixed episode, where neurochemical abnormalities may be more pronounced.
Methods: We recruited 11 BD patients (8 in manic, 3 in mixed episodes; age 37.1±12.0; 4 males) and 17 healthy controls (age 29.9±8.0; 8 males) with no medical/neurological problems and no substance abuse (age difference $p=0.07$). The patients were all hospitalized, on mood stabilizing medications, and had a mean Young Mania Rating Scale score of 25 at the time of the scan. Three patients were having a first manic episode. Following provision of informed consent, the patients underwent 2D J-resolved proton MRS scanning in a 4T Varian scanner. This type of MRS permits the simultaneous quantification of coupled spins (e.g. GABA, Glu) as well as uncoupled spins (e.g. Creatine, Choline etc.). We recently used this approach to quantify Glu and GABA simulatenously in clinically acceptable scan times on ten healthy controls scanned three times. The coefficient of variation in that study was 10.9% for Glu and 39.5% for GABA. In the current study, we acquired data from two 2x2x2cm voxels, one in the pregenual anterior cingulate cortex (ACC) and one in the parieto-occipital cortex (POC). The metabolite concentrations were quantified

using LCModel with simulated J-resolved basis sets and are presented as metabolite:Creatine ratios. Data points with a Cramer-Rao Lower Bound of >50% were excluded from the analysis, as were any spectra where signal:noise was <13. Grey and white matter (GM and WM) and cerebrospinal fluid (CSF) distributions in each voxel were quantified using T1 weighted images.
Results: There were no group differences in GM, WM and CSF composition of study voxels. Creatine levels were also comparable across groups. We found a reduction in Glu levels in the ACC (11%) and POC (18%) in the BD group compared to control ($F(15,1):10.31$, $p=0.006$; no effect of brain region, no significant brain region x diagnosis interaction). In contrast, there was an elevation in GABA levels in the ACC (33%) and POC (34%) in the BD group compared to control but there was more variability in this measure ($F(14,1):4.78$, $p=0.046$; no effect of brain region, no significant brain region x diagnosis interaction). We also found significant reductions in NAA in both brain regions. There were no significant group differences or brain region x diagnosis interactions in Choline and myo-Inositol levels in repeated measures ANOVA, although myo-Inositol was reduced by 19% in the ACC voxel in BD ($t:2.34$, $p=0.028$).
Discussion: We found a significant reduction in Glu and elevation in GABA levels in the ACC and POC in acutely manic or mixed BD patients. These findings indicate the presence of significant abnormalities in synaptic neurotransmission in the cerebral cortex in acute mania, consistent with glial and neuronal abnormalities identified in postmortem studies of BD. The pattern of findings is the converse of what has previously been reported in major depressive disorder (Sanacora et al, 2004) and in bipolar depression (Dager et al, 2004). A major limitation of our findings is that the patients were taking medications at scan time. However, we scanned acutely ill patients, in whom medications were recently instituted and before they were effective. We also replicated the NAA reduction previously reported in medication-free BD subjects, arguing against a significant medication effect here.

97. Anxiety-Like Effects of Corticotropin-Releasing Factor (CRF) Are Reduced by 6-Hydroxydopamine Lesions of the Bed Nucleus of the Stria Terminalis (BNST)

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Background: The neuropeptide corticotropin-releasing factor (CRF) may play a role in the pathophysiology of anxiety disorders. In particular, extrahypothalamic sources of CRF, such as the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST) are known to be involved in the generation of anxiety-like behaviors. As such, dysfunction within CRF brain circuits, a condition that has been putatively linked to chronic stress exposure, may underlie the development and manifestation of anxiety-related disorders. Several studies have shown that the interaction between norepinephrine (NE) and CRF systems might be an important component in adaptive and maladaptive responses to stress (Koob, 1999). Less is known about the interaction between dopamine (DA) and CRF in mediating stress and anxiety-like effects. Potential sites of convergence between these systems exist, however, with the dorsolateral BNST (dlBNST). The dlBNST has a high density of CRF-containing neurons and receives heavy dopaminergic inputs that make direct synaptic contact with CRF neurons (Phelix et al., 1994). The BNST has also been shown to be a critical brain area mediating CRF-enhanced startle (Lee & Davis, 1997), a behavioral assay believed to reflect stress- or anxiety-like states. We have shown previously that dopamine D1 receptor-mediated neurotransmission is involved in the expression of CRF-enhanced startle, and that a dopaminergic projection from A10dc neurons in the periaqueductal gray (PAG) to the dlBNST may mediate this effect (Meloni et al., 2006). In the present study, we examined the effect of lesioning the dopaminergic projection from the A10dc/PAG to the dlBNST on CRF-enhanced startle.
Methods: Male Sprague-Dawley rats (400 g) were matched into two groups having equivalent levels of baseline startle. Two days later, groups of rats received bilateral infusion of either vehicle (0.9% saline; SHAM group) or the neurotoxin 6-hydroxydopamine (6-OHDA; 2.5 ug/0.3 ul/side) into the dlBNST. All animals were also implanted with infusion cannulas (23-gauge) into the lateral ventricle for intracerebroventricular (icv) delivery of CRF. Two weeks later, animals received icv CRF (1 ug), were placed in the test cages, and the amplitude of their startle reflex was measured in response to 400 startle-eliciting acoustic stimuli at each of three different intensities (95, 100, 105 dB; 30-s interstimulus interval). Tyrosine hydroxylase (TH) immunohistochemistry was performed on brain sections through the BNST and A10dc/PAG area to determine the extent of the 6-OHDA-induced lesions.
Results: 6-OHDA dlBNST lesions had no effect on baseline startle but significantly reduced the anxiety-like effects of CRF on startle across the entire test session. TH immunohistochemistry showed that 6-OHDA produced almost complete destruction (>95 %) of TH fibers in the dlBNST and significantly reduced the number of TH-positive neurons in the A10dc/PAG area.
Discussion: These data suggest that neuronal regulation of the dlBNST by dopamine may be an important mechanism for controlling CRF-dependent moods and affective states. If true, an imbalance or breakdown in the allostatic state between these two systems within the extended amygdala (e.g. CeA and/or BNST) could play a role in the pathophysiology of anxiety disorders. We hypothesize that the dopaminergic projection from the A10dc/PAG area to the dlBNST may be particularly susceptible to the deleterious effects of chronic stress exposure, subsequently leading to a heightened state of anxiety.

98. Sex Differences in Response to Metyrapone in Patients with Major Depression

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Background: Major depression is accompanied by increased activation of the hypothalamic pituitary adrenal (HPA) axis. Our previous studies indicated that increased ACTH secretion in response to metyrapone in patients with depression occurred between 4PM-10PM. To determine if this increased drive was related to increased activation around the clock or was specific to the afternoon/evening time frame, we conducted studies over 24H with metyrapone blockade.
Methods: We recruited 28 drug free patients with major depression and 28 age and sex matched control subjects. Metyrapone, 750 mg was given q4h starting at 4PM and continuing through 12noon the following day. Blood was drawn every 10 minutes to measure ACTH concentration for 24H beginning at 4PM of day 1 and ending 4PM day 2.
Results: In depressed women we saw increased ACTH secretion between 4PM and 10PM but normal peak ACTH secretion compared to matched control women under metyrapone blockade. In depressed men we observed decreased 4PM -10PM ACTH secretion and decreased maximal ACTH response to metyrapone compared to matched controls or depredsed women. However, depressed men demonstrated increased number of ACTH pulses compared to matched control men while ACTH pulsatility was normal in depressed women.
Discussion: Men and women may show different patterns of ACTH dysregulation in major depression. Increased activation in the evening under metyrapone challenge appears to be specific to depressed women in the two studies we conducted that included both male and female patients. ACTH pulse number is an additional indicator of central neuroendocrine systems controlling CRH/ACTH release i.e. the CRH pulse generator. The greater number of ACTH pulses in men indicate a different type of dysregulation of the axis in depressed men. This greater number of ACTH pulses in depressed

men agree with the data of Deuschle et al examing ACTH pulsatility in depressed men under basal endocrine conditions. Supported by NIMH MH50030 to Elizabeth A Young

99. Reduction of Central Neural Response to Motivating Stimuli: A Common Effect of Models of Depression

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Sponsor: Murray Alpert

Background: One of the defining characteristics of major depression is a stark loss of positively motivated active behaviors. It has been proposed that this symptom is the result of reduced neural activity in brain networks involved in motivated activities. The present experiment was designed to test this hypothesis by measuring the effect of experimentally induced depression in mice on the ability of motivating stimuli to induce neural activity in brain regions involved in behavioral activation. The ability of several antidepressant agents to reverse any changes was also examined.
Methods: Experimental depression was established in mice by 5 separate paradigms: acute immune activation by lipopolysaccharide; subacute monoamine depletion by reserpine; repeated forced swim stress; acute intraventricular injection of galanin; and chronic subordination stress. The motivating stimuli used to challenge the depressed animals were either a 15 min swim in a large tank of warm water or a 90 min exposure to a novel (fresh) home cage. Active behaviors were recorded on videotape during these procedures. Neural activity was assessed from the expression of c-fos or phosphorylation of mitogen-activated protein kinase (MAPK) in 5 brain regions, 4 of which are known to be associated with positive active behavior (nucleus accumbens, NAC; secondary motor cortex, M2; posterior cingulate gyrus, CG; anterior piriform cortex, APIR) and one of which with stress reactions (paraventricular hypothalamus, PVH). The antidepressants, given as chronic pretreatments, were desmethylimipramine, tranylcypromine, escitalopram or environmental enrichment, a nonpharmacological procedure recently shown to have antidepressant properties.
Results: It was found that all of the depression models reduced fos expression and MAPK activation responses in the 4 activational brain regions (NAC, M2, CG, APIR) but either did not effect or increased the response of the stress-associated region (PVH). Chronic pretreatment with desmethylimipramine, tranylcypromine, escitalopram or environmental enrichment attenuated or blocked these effects.
Discussion: These findings support the hypothesis that reduced motivated behavioral activity in mouse depression models is associated with and probably caused by a reduced neural activity in positive activational regions in the CNS that may be secondary to the activation of central stress circuits. These changes appear similar to those occurring in human depression.

100. Macromolecular White Matter Abnormalities in Geriatric Depression: A Magnetization Transfer Imaging Study

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Sponsor: Robert C. Young

Background: Geriatric depression consists of complex and heterogeneous behaviors unlikely to be caused by a single brain lesion. However, there is evidence that abnormalities in specific brain structures and their interconnections confer vulnerability to the development of late-life depression. To assess the presence of structural brain abnormalities in geriatric depression, we used magnetization transfer (MT) in older depressed patients and age-matched control participants. MT imaging provides contrast based on the interaction of normally observed tissue water signal with protons bound by large macromolecules, including myelin. The magnetization transfer ratio (MTR) is a measure of the difference in signal between free and macromolecule-bound water, and it is believed to be an index of myelin integrity. This study tested the hypothesis that depressed elders would exhibit lower MTR in frontostritatal and limbic regions than age-matched control subjects.
Methods: Sixty-one subjects (age>60 years) with major depression (by SCID, DSM-IV) and a Hamilton Depression Rating Scale (HDRS) score of 19 or higher and 24 age-matched, non-psychiatric comparison subjects completed an MRI scan. To determine the MTR, a 3D FLASH sequence was acquired both with and without an off-resonance magnetization pulse (TR = 31 ms, TE = 6 ms, 160x256 matrix (5/8 reduced), FOV=320 mm, 32 slices, 5 mm slice thickness, 0 mm skip). The identical sequence was repeated with an off-resonance pulse with a 1.5 kHz frequency offset. MTR was computed as the percent intensity difference between the on- and off-resonance pulse sequences. Images were normalized to Talairach space. Voxel-based analysis of MTR data was conducted with a general linear model using age as the covariate. To control for type I error a threshold was used that allows only clusters of at least 100 voxels, all significant at $p < .05$. In addition, at least one of the voxels in the cluster had to be significant at $p < .005$.
Results: The patients and controls did not differ in age or MMSE performance. Relative to controls, patients demonstrated lower MTR in multiple frontolimbic regions, including white matter of the left subcallosal, dorsal anterior cingulate, inferior and middle frontal, insular, and posterior cingulate regions. Depressed patients also had lower MTR in left thalamic, perirolandic, and precuneus regions, and in the left splenium of the corpus callosum.
Discussion: To our knowledge, this is the first report of lower MTR in frontolimbic regions of older patients with major depression compared to age-matched controls. These findings complement diffusion tensor studies of geriatric depression that indicate the presence of fractional anisotropy abnormalities in select frontostriatal and limbic regions that correlate with executive dysfunction and predict treatment response.

101. Genetic Variant BDNF (Val66Met) Polymorphism Alters Anxiety-Related Behavior

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Sponsor: James H. Kocsis

Background: A common single nucleotide polymorphism (SNP) in the brain-derived neurotrophic factor gene (Val66Met) is associated with alterations in brain anatomy and memory, but its relevance to clinical disorders is unclear.
Methods: We generated a transgenic mouse in which the variant BDNFMet is endogenously expressed, by designing a BDNFMet knock-in allele in which transcription of BDNFMet is regulated by endogenous BDNF promoters. Analyses of this transgenic mouse model involved biochemical studies of BDNF secretion from neurons obtained from BDNFMet mice, as well as anatomical and behavioral studies.
Results: In this transgenic knock-in mouse, variant BDNFMet was expressed in brain at normal levels, but its secretion from neurons was defective. The variant BDNF mouse reproduced the phenotypic hallmarks in humans with the variant allele: altered hippocampal volume and impairment in hippocampal-dependent memory. Our subsequent analyses of these mice elucidated a phenotype that had not been established in human carriers: increased anxiety. When placed in conflict settings, BDNFMet mice display increased anxiety-related behaviors in 3 separate anxiety-related behavioral tests. Finally, the

form of anxiety elicited in these BDNFMet mice was not responsive to a common SSRI, fluoxetine.
Discussion: These findings indicate that an allelic variant BDNF may play a key role in the genetic predisposition in humans to anxiety and depressive disorders, as well as differential response to standard antidepressant treatment.

102. Characterizing and Predicting Major Depression Following Pegylated Interferon-Alpha Administration

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Sponsor: Past Travel Awardee, NIMH, 2004

Background: A bidirectional relationship between immunologic abnormalities and mood disorders is becoming increasingly explicated. Exogenous interferon-alpha2 (IFN), an inflammatory cytokine, can trigger a major depressive episode in euthymic individuals, an effect that may offer the opportunity to prospectively examine the development of major depression. However, the specific nature of IFN's psychiatric effects requires further characterization.
Methods: We prospectively examined twenty-three euthymic adults, age 20 to 58; evaluating subjects at baseline and then during weekly IFN treatment using both the Structured Clinical Interview for DSM-IV and self-report questionnaires. Potentially confounding factors such as concurrent psychiatric medications and active psychiatric or neurological disorders were excluded. Levels of C-Reactive Protein (CRP) and IL-6 were prospectively measured. Principal component (PC) analysis with varimax rotation was used to examine changes in questionnaire scores.
Results: Major depression (MDD) developed in 39% of subjects within 3 months. After one month of treatment, the Beck Depression Inventory (BDI) increased from 4.6 +/- 4.4 to 9.7 +/- 6.3 ($t(22)=5.4$; $p<0.005$) and hostility also increased from 0.2 +/- 0.25 to 0.58 +/- 0.55 ($t(17)=3.0$; $p<.05$). PC analysis of changes following IFN treatment demonstrated three distinct orthogonal factors: (i) depression-specific symptoms, (ii) hostility and anxiety, and (iii) generalized distress that included a combination of somatic symptoms and nonspecific depression symptoms. BDI at one month was solely predicted by pre-treatment BDI ($r=0.76$, $p<0.01$). Hostility at one month was predicted by pre-treatment agreeableness ($r=0.75$, $p<0.001$), but not baseline hostility. Increased hostility also correlated with the change in CRP levels ($r=0.65$, $p<0.05$). Categorical MDD was predicted by a combination of pre-treatment BDI scores, pre-treatment neuroticism, and agreeableness (combined $r=0.66$, $p<0.05$). The change in the ratio of CRP to IL-6 strongly correlated with the development of MDD.
Discussion: These results indicate that (i) the categorical MDD induced by IFN may reflect a combination of increased BDI symptoms and increased hostility, (ii) some individuals are independently vulnerable to worsened hostility, (iii) peripheral effects on other inflammatory biomarkers correlate with psychiatric effects, and (iv) it may be possible to identify vulnerabilities even in initially euthymic subjects.

103. Can Treatment Response to Antipsychotic Drug Be Predicted in Schizophrenia?

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Sponsor: William Carpenter

Background: Using PET with ^{15}O, we sought to evaluate whether drug-free and antipsychotic-induced rCBF patterns would be predictive of treatment response in patients with schizophrenia. Based on our previous work, we had hypothesized that drug-induced rCBF changes in limbic regions would predict treatment response.
Methods: Patients with schizophrenia were initially scanned during a resting state after withdrawal of all psychotropic medication (two weeks), and then blindly randomized to treatment with haloperidol (Hal)(n=12) or olanzapine (Olanz)(n=17) for a period of 6 weeks. Patients were scanned again after 1 and 6 weeks of treatment. All assessments, including scanning sessions, were obtained in a double-blind manner. To evaluate if drug-free rCBF patterns were predictive of treatment response, we generated pixel-by-pixel correlations between the drug-free scans and the BPRS Psychosis change scores after 6 weeks of treatment. Likewise, to evaluate whether rCBF changes after one week would predict treatment response, we generated correlations between the rCBF changes after one week and the BPRS Psychosis change scores at the end of treatment.
Results: There was a significant correlation ($r=0.4$, $p=0.04$) between rCBF in the right ventral striatum and the BPRS Psychosis change scores with treatment. Low ventral striatum in drug-free subjects was predictive of good treatment response. In both treatment groups, there were significant correlations between rCBF changes in both the ventral striatum (Olanz, $r=-0.76$, $p=0.01$, Hal, $r=-0.85$, $p=0.01$) and the hippocampus (Olanz, $r=0.72$, $p=0.01$, Hal, $r=0.79$, $p=0.02$) and the BPRS Psychosis change scores with treatment. Increased ventral striatum and decreased hippocampal activity as the result of one week of treatment were predictive of treatment response.
Discussion: Blockade of dopamine D2 receptors in the ventral striatum is a likely first step into antipsychotic action and appears to be followed by neuronal events affecting limbic regions. We propose that treatment response is characterized by an improved neuronal transmission starting in the ventral striatum and relayed to limbic regions through efferent projections. In conclusion, rCBF patterns in ventral striatum and hippocampus may represent imaging markers indexing treatment response to psychosis. The ability to predict treatment response has the potential to hasten therapeutic decisions. A clear understanding of the biological correlates of treatment response to positive symptoms could provide a solid base for the development of more effective and specifically targeted drugs. Supported by RO1 MH57971 and the University of Maryland GCRC MO1 RR 16500

104. Prenatal Infection and Executive Dysfunction in Adult Schizophrenia

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Sponsor: Jean Endicott

Background: In our previous studies,we demonstrated significant associations between serologically documented prenatal exposure to infection and risk of adult schizophrenia. In the present study, we aimed to assess whether these in utero infections were associated with abnormalities in executive function in patients with schizophrenia.
Methods: We conducted assessments of executive function in 52 subjects derived from a large Northern California cohort born from 1959 to 1967 and followed up for schizophrenia. Subjects were classified previously as having been exposed or unexposed to prenatal influenza or toxoplasmosis by analyses of archived maternal sera.
Results: We found that schizophrenia cases who had been exposed to prenatal infection, compared to unexposed cases, had significantly greater total errors on the Wisconsin Card Sort Test (exposed: mean (SD)=21.00 (15.72); unexposed: mean (SD)=12.28 (8.48); $t=2.10$, $p=.047$), and required a significantly longer time (secs.) to complete the Trails B test (exposed: mean (S.D.)=142.71 (66.55); unexposed: mean=87.63 (25.41); $t=3.12$, $p=.005$). There were no significant differences between the groups on performance on the Letter Number Sequencing Test or Digit Symbol tests, which also require working memory but not set shifting, or on tests of psychomotor or processing speed, including Trails A.

Discussion: Prenatal infection is associated with deficits in set shifting in adult patients with schizophrenia. These results suggest that prospectively measured in utero infection is associated with abnormalities in specific aspects of executive function in schizophrenia.

105. First Episode Psychosis and Substance Abuse: A Two-Year Efficacy Trial of Olanzapine vs. Haloperidol

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Background: Our group has recently completed a two year study of olanzapine and haloperidol in patients with first episode psychosis. In this study, olanzapine had some small advantages over haloperidol in acute therapeutic response, in two year rates of medication discontinuation, and in neurological side effects as compared to treatment with haloperidol, although it was associated with more weight gain and an increased cholesterol level. In this sample of first episode patients, 37% had a lifetime diagnosis of substance use disorder (SUD). Patients with SUD were more likely to be men, to have more positive symptoms and fewer negative symptoms than those without SUD at study entry, and those with cannabis use disorder (CUD) had an earlier age of onset. Moreover, those with SUD at baseline had a generally poorer response to treatment over the first 12 weeks of treatment with either olanzapine or haloperidol. Here, we describe a post-hoc analysis of the effects of co-occurring SUD on outcome over the full course of the two year study.
Methods: The study was a double-blind, randomized, investigation of olanzapine vs. haloperidol in 263 patients (ages 16 to 40) with first episode psychosis (meeting DSM-IV criteria for schizophrenia, schizoaffective disorder or schizophreniform disorder). Patients had not been psychotic for longer than 5 years, and had no more than 16 cumulative weeks of treatment with an antipsychotic medication. The dose ranges for olanzapine and haloperidol during the study were 5 – 20 mg/day and 2 – 20 mg/day, respectively. Psychopathology was assessed by the PANSS, the MADRS and the CGI. Responders were identified by: (1) no rating of >3 (mild) on items P1, P2, P3, P5 and P6 of the PANSS; and (2) CGI severity score <4 (moderately ill). Substance use disorder (SUD) was assessed at baseline (with the SCID) to determine whether there was a lifetime history or current evidence of alcohol or other substance use disorder (abuse or dependence). Information was available for substance (including alcohol) use disorder (SUD), alcohol use disorder (AUD), and cannabis use disorder (CUD).
Results: Patients with SUD (as compared to those without SUD) were less likely to respond to treatment (56.3% vs. 72.4%; Fisher Exact P< 0.0094). If analyzed by treatment group, this difference was significant for the subgroup of patients treated with haloperidol, but not olanzapine. If assessed by substance and treatment group, the difference in response was confined to those with a history of CUD who were treated with haloperidol. Improvement in PANSS total (and PANSS positive symptoms) was less in patients with a SUD diagnosis than in patients without such a diagnosis. Among patients with AUD, change in PANSS total (and PANSS negative symptoms) was less than among those without SUD; among patients with CUD, improvements in PANSS total and PANSS positive were less than in those without SUD. Assessing the number of days to study discontinuation, there was a trend for patients with SUD to stay in the study for a shorter time than those without SUD. This difference was significant for patients with a CUD diagnosis (p=.0216). When the time in study was assessed by treatment medication, it appeared that the difference of time in study among those with SUD or CUD was confined to the haloperidol group, such that there was a trend to shorter time in study in those with SUD (p=.06) and a significantly shorter time in the study for the CUD group (p=.006).
Discussion: A post-hoc analysis of data from a randomized controlled trial of olanzapine vs. haloperidol in patients in their first episode of psychosis suggests that the benefits provided by olanzapine as compared to haloperidol appear to occur largely in patients with a co-occurring SUD, particularly those with CUD.

106. A Non-Selective D3/D2 Antagonist but not a Selective D3 Antagonist is Active in Rodent Models Predictive of Antipsychotic Activity

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Sponsor: Athina Markou

Background: The restrictive and selective expression of dopamine D3 receptors in the mesolimbic dopaminergic system has led to the hypothesis that antagonism of these receptors has antipsychotic efficacy in the absence of motor side effects, such as extrapyramidal symptoms. This study aimed to evaluate the activity of dopamine D3 antagonist compounds in rodent models mimicking positive and negative symptoms of schizophrenia.
Methods: The in vitro affinity, ex vivo receptor occupancy, and activity in animal models, was determined for the GSK derivative SB-277011 and the 2 enantiomers of a chemotype published by Servier, that we refer to as S33138a and S33138b.
Results: Our in vitro data show D3 over D2 selectivity (ratio of Ki values) of a factor 190 for SB277011 (Ki values of 9 and 1730 nM respectively), and a factor 37 and 30 for both enantiomers of S33138 (Ki values of 28 and 1030 nM respectively for S33138a and 12 and 360 nM respectively for S33138b). Ex vivo receptor occupancy studies in rat brain revealed that SB277011 preferentially occupies D3 receptors over D2 receptors (ED50: 2.1 mg/kg and >40 mg/kg s.c. respectively), whereas dose response curves for both enantiomers of S33138 showed little preference for D3 over D2 (ED50: 26 and >40 mg/kg s.c. respectively for S33138a; ED50: >10 and >10 mg/kg s.c., respectively, for S33138b). Our data further illustrate the effects of SB277011 and S33138a in various animal models currently used for characterizing antipsychotics: antagonism of dopamine stimulant-induced behavior, antagonism of phencyclidine-induced hyperactivity and social interaction deficit. In addition, motor side-effect liability was assessed in catalepsy models. SB277011 did not inhibit apomorphine or D-amphetamine-induced behavior, nor did it antagonize PCP-induced hyperactivity in rodent, at doses of at least 4 times and more the ED50 for D3 occupancy, indicating little activity of selective D3 antagonism in these models. The less selective compound S33138a showed activity in the apomorphine and the amphetamine tests (ED50 5 mg/kg and 20 mg/kg respectively) but was not active against PCP-induced hyperactivity. The activity observed with S33138a in these models could be due to either D3 or D2 antagonism. A dose of 20 mg/kg SB277011 further reduced the time in social interaction in a PCP-disrupted social interaction test, while S33138 showed no effect in this model.
Discussion: We conclude that above rodent models are not sensitive enough to detect D3 antagonist activity. Moreover, activity in these models is confounded by additional D2 activity.

107. The Effect of Transdermal Nicotine on Episodic Memory Performance in Non-Smoking Patients with Schizophrenia

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Cognitive deficits, including impaired verbal memory, are a significant component of the schizophrenia syndrome and a strong predictor of functional outcome for these patients.

Although there are no FDA-approved treatments for the cognitive deficits associated with this illness, several agents are under intense investigation, including nicotine. This study utilized a randomized, placebo-controlled, double-blind, crossover design to investigate the effects of transdermal nicotine on episodic memory performance.
Methods: Ten non-smoking patients with schizophrenia and twelve non-smoking healthy control subjects completed the study. At each of two study visits, subjects performed a memory test both before and after application of a patch containing either nicotine or placebo. The memory test involved hearing words spoken by a male or female voice and then after a brief delay, attempting to recall whether visually presented words had been said by the male, the female, or were unpresented novel foils. There were three primary outcome measures: 1) Hit rate (percentage of old items correctly identified as old); 2) False alarm rate (percentage of new words incorrectly labeled as "old"); and 3) Source memory accuracy (percentage of old items attributed to their correct source (male or female)). For each measure, pre-patch scores were subtracted from their associated post-patch scores, and these difference values were then entered into an analysis of variance with group (control vs. schizophrenia) as a between-subjects effect and treatment (nicotine vs. placebo) as a within subjects effect.
Results: When compared to placebo, nicotine had no significant effect on the accuracy of response to old items (hit rate), but did lead to a significantly reduced false alarm rate in all subjects (main effect of treatment: $F(1,18)=9.14$, $p=0.007$). In addition, there was a trend-level interaction between treatment and group ($F(1,18)=3.29$; $p=0.086$), as nicotine led to a greater reduction in false alarms in patients with schizophrenia (mean pre-post nicotine change = -8.7 + 11.0) than in the control subjects (mean pre-post nicotine change = -2.1 + 3.8). When the responses to new and old items were considered together, there was no overall evidence for an effect of nicotine on overall memory accuracy (as defined by d', discriminability), nor was there an effect of nicotine on source memory accuracy.
Discussion: Taken together, these results demonstrate no effect of nicotine in enhancing the recognition or retrieval of previously experienced events. Nicotine does appear, however, to lead to improvement in the correct identification of novel events. Although this enhancement in "novelty detection" occurred in both groups, the effect may be stronger in patients with schizophrenia. Given the high rates of false alarms in these patients, and the potential link between false alarms and aspects of schizophrenic phenomenology (e.g., hallucinations and delusions), this finding may be of important clinical significance.

108. Habituation Deficits Induced by mGlu2/3 Receptor Blockade in Mice: Reversal by Antipsychotic Drugs

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Sponsor: Past Travel Awardee, Memorial, 2003

Background: Cortical metabotropic glutamate receptors (mGluR) appear to be involved in habituation of simple stimulus-bound behaviors. Habituation deficits may also affect more complex behaviors, resulting in lack of behavioral flexibility, and may be involved in the pathophysiology of schizophrenia.
Methods: In the present study, male CD1 and NMRI mice were injected with the mGluR2/3 antagonist, LY341495, prior to being placed into the novel arenas for automatic motor activity recording (two-hour sessions).
Results: Administration of LY341495 (1-30 mg/kg) dose-dependently prevented the habituation of the locomotor activity. LY341495 (10 mg/kg)-induced habituation deficit was fully and dose-dependently suppressed by haloperidol (0.03-0.3 mg/kg), clozapine (1-10 mg/kg), risperidone (0.01-0.1 mg/kg), aripiprazole (1-10 mg/kg), and sulpiride (3-30 mg/kg). For all of these agents (except for haloperidol), the effect of LY341495 was attenuated at the dose levels that did not affect spontaneous motor activity. Application of imipramine, diazepam, pentobarbital, as well as several agonists and / or antagonists acting at various receptor systems (e.g., 5-HT1A, 5-HT2A, 5-HT3, 5-HT6, D4, CB1, AMPA/glutamate) had no appreciable effects.
Discussion: Thus, antipsychotic drugs selectively reverse the behavioral deficits induced by mGluR2/3 antagonist.

109. Family History of Affective versus Psychotic Disorder Influences Symptom Expression in Schizophrenia

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Sponsor: Travel Awardee, Pfizer, 2006

Background: Schizophrenia is a heterogeneous disorder with a strong genetic component. Interestingly, there is a considerable amount of overlap in genes that have been associated with schizophrenia and bipolar disorder. Patients with an affective diagnosis in addition to schizophrenia have a better course of illness. However, it is not known whether a family history of affective illness also improves the course of illness for schizophrenia patients. In this study, we examined clinical and demographic background characteristics and current symptomatology in patients with schizophrenia with respect to family history of affective disorders and non-affective psychoses.
Methods: Family histories were determined for 398 patients enrolled from the NYSPI Schizophrenia Research Unit by trained raters with high IRR. Family informants also provided clinical information about 1st and 2nd degree family members. We defined four family groups of our cases by family history of affective disorder, non-affective psychosis, both or neither. Symptom assessments and demographic information were collected on a subsample of these patients by researchers blind to patient identifiers. The Positive and Negative Syndrome Scale (PANSS) was the primary outcome measure and was separated into the five factors, which were analyzed across the family history groups covarying for demographic factors.
Results: The family history groups did not differ in gender distribution, age, age at onset of first symptoms, age at first treatment, or in global assessments of function during the worse period in the current episode or in the last month. However they did differ in education such that patients with a family history of affective illness completed more years of education than the patients with a family history of schizophrenia and with no family history of either illness. We found differences between the family history groups on the PANSS activation factor ($F(3, 213) = 3.73$, $p=.012$). In particular, patients with a family history of affective disorder had lower scores than those with a family history of non-affective psychosis. In addition there was an effect of gender across all groups on the activation factor ($F(1, 213) = 9.61$, $p=.002$) with women ($M=10.09$, $SD=4.87$) scoring higher than men ($M=8.81$, $SD=3.56$); and for the positive subscale factor scores ($F(1, 213) = 8.07$, $p=.005$), also with women ($M=15.22$, $SD=5.83$) scoring higher than men ($M=13.22$, $SD=5.81$). There was no interaction between family history group and gender. We also did not find any significant differences between the family history groups on overall pattern and severity of symptoms.
Discussion: We found that among schizophrenia patients, having a family history of affective disorder may lessen excitability. Such differences in symptom expression may thereby define a distinct subset of schizophrenia patients. Although subsets of patients with different genetic heritability may present similarly, differences in symptom severity may represent a unique biology based on genetic differences.

110. Treatment of the Schizophrenia Prodrome: Do Antidepressants Work?

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Sponsor: Daniel Javitt

Background: In parallel with other types of chronic illness, an interest in the possible prevention of schizophrenia has increased over the past decade. As a result, there has been a dramatic escalation in studies assessing the extent to which treatment with antipsychotics, initiated prior to the onset of psychosis, might prevent psychosis from emerging. Initial findings have been encouraging but not definitive and have raised a number of important questions, among these whether antipsychotic treatment is the only, or even optimal, early intervention. The current study, which has been conducted as part of the Recognition and Prevention (RAP) program in New York, was concerned with possible alternate pharmacological treatments, and, in particular, with the potential of antidepressants for early intervention.
Methods: Forty-eight adolescents (mean age: 15.8 years) participated in a naturalistic medication study as part of the initial research phase (2000-2005) of the RAP program. All participating adolescents were treatment-seeking and considered to be in the prodromal phase of schizophrenia. Individuals were selected from the overall Phase I sample (n=152) if they had: 1) displayed attenuated positive symptoms at a moderate to severe level; 2) been treated pharmacologically for at least eight weeks; 3) had been followed-up for at least six months (mean follow-up=30.5 months) and 4) had sufficient information available to rate adherence to medication. Psychiatrists, blind to research hypotheses and ratings, prescribed treatment based on best practice standards.
Results: Two types of medication were naturalistically prescribed: antidepressants (ADP, n=20) or second generation antipsychotics (SGAP, n=28), with polypharmacy common. There were no significant differences in presenting symptoms at baseline between subjects receiving SGAPs vs ADPs on either positive, negative, disorganized or general symptoms. The only exception was disorganized thinking, which was more severe in SGAP-treated adolescents. Twelve of the 48 adolescents (25%) developed a psychotic disorder over the follow-up period (highest conversion rate during year 2 of follow-up), with all converters having been prescribed SGAPs. There were no conversions among ADP-treated adolescents, with conversions thus significantly lower than for SGAP treated adolescents (logrank $\chi 2=7.36$, df=1; p=.007). Treatment outcome, however, was confounded, since 11 of the 12 converters were non-adherent. Adolescents, in general, were more likely to be non-adherent to SGAPs (61%) than to ADPs (20%; $\chi 2=7.86$; p=.005). Improvement in total positive symptoms over time was significant (F=18.08, p<.0001) and similar for both medications (time x medication was not significantly different). Disorganized thinking, however, did not improve regardless of treatment.
Discussion: The findings reported are primarily hypothesis generating, given non-random assignment and the possibility that some proportion of prodromal subjects responding to ADPs may, in the long run, prove to be false positives. However, it can be stated at this point that, with follow-up, a number of adolescents meeting criteria for prodromal schizophrenia were successfully treated with antidepressants. Thus these findings suggest that, in some cases, it might be preferable to begin treatment with antidepressants, and progress to antipsychotics once symptoms intensify, since adherence to anti-psychotics is more difficult to maintain.

111. Association Between G72/G30 D-amino Acid Oxidase Activator and Longitudinal MRI Brain Volume Reductions in Schizophrenia

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Background: Variability in brain volumes can in part be attributed to genetic factors. Recent studies indicate that polymorphisms from an increasingly number of genes (e.g. BDNF, DISC1, COMT, ASPM) account for some of the variance in MRI brain volumes among healthy volunteers as well as in schizophrenia patients. Since schizophrenia is associated with progressive frontotemporal brain volume reductions following illness onset, understanding the genetic factors contributing to longitudinal brain volume changes may help advance our knowledge regarding the neurobiology of schizophrenia.
Methods: In this study, we examined the association between a SNP from the G72/G30 locus on Chromosome 13q (rs3916965) and MRI brain volume changes in 109 schizophrenia patients (Mean interscan interval=3 years). The G72 protein activates D-amino acid oxidase, an enzyme involved in the metabolism of D-serine, which in turn, is an agonist at the glycine modulation site of the NMDA receptor. Various SNPs in the G72 gene have been linked with schizophrenia, including rs3916965-A-allele over-transmission.
Results: Using multivariate repeated measures analysis of covariance, we found significant genotype main effects on within-subject frontal gray matter (GM) and frontal white matter (WM) brain volume changes ($F \geq 4.28$, df=1,104, $p \leq 0.04$). AA homozygous patients showed significant frontal GM and WM volume reductions while mean frontal brain volumes among G-allele carriers remained stable over time ($T \geq 2.20$, df=107, $p \leq 0.03$). No statistically significant genotype effects were found on within-subject changes in temporal or parietal brain volumes.
Discussion: These findings suggest that the A-allele may be a mediator of progressive frontal brain volume reductions in schizophrenia. Although effects of the A-allele on glutamate neurotransmission are unknown, this SNP could, through G72 protein's role in activating D-amino acid oxidase, modulate NMDA receptor signaling, contribute to glutamatergic toxicity and progressive frontal brain volume decrement in schizophrenia.

112. Bipolar Disorder with Psychotic Symptoms Shows Significant Linkage to Chromosome 5q in the Area of the GABA-Receptor Cluster

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Sponsor: Murray Jarvik

Background: Genetic approaches such as linkage and association studies have promised to shed some light on the underlying pathomechanisms of bipolar disorder through the identification of risk factors that may contribute to the manifestations of this disorder. However, to date only very few if any genetic loci have been identified unambiguously through replication of a significant linkage signal. The heterogeneity of the phenotype may be one of the contributing factors to this problem. Bipolar disorder with psychotic symptoms has recently emerged as a possible subtype of mood disorder with specific genetic risk factors. Several groups have reported suggestive linkage signals between this phenotype and an area on chromosome 5q that has been also suggested in linkage scans for schizophrenia. The identification of a risk carrying sequence variation in this chromosomal area would help to clarify the diagnostic boundaries between bipolar disorder or a subtype of it and schizophrenia.
Methods: In our re-analysis of the National Institute of Mental Health Bipolar Genetics Initiative data sets we selected families in which at least two members were affected with psychotic bipolar disorder. We found a significant linkage signal (nonparametric LOD score of 5.0) in 41 families (381 individuals) to a 1 centi Morgan region on the long arm of chromosome 5 using the program MENDEL (Kerner B, 2006). This area contains among others clusters of GABA receptor subtype genes. We genotyped 1134 single nucleotide polymorphisms near the linkage peak in those families with evidence for linkage. In a family based approach we then tested for association between the polymorphisms and the trait in the presence of linkage using the FBAT package.

Results: The most significant signal in a preliminary analysis was found at marker rs12658202 (Z-score = 3.673, p=0.00024 in 30 families uncorrected for the multiple tests performed). We found evidence for allelic heterogeneity.
Discussion: The identification of nucleotide polymorphisms that are closely linked to the bipolar phenotype or a specific symptom of it is of utmost importance for the ultimate identification of risk associated DNA sequence variations. Those sequence variations could be located in a coding sequence or a regulatory sequence. The identification of risk alleles for bipolar disorder will ultimately help to better understand the pathophysiology of the disorder and its diagnostic boundaries to other psychiatric disorders. It may also facilitate the development of more specific and effective treatment options.

113. Association of D5 Dopamine Receptors with Pre- and Postsynaptic Elements of Glutamatergic Synapses

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Sponsor: Frank D. Yocca

Background: Dopaminergic neurotransmission mediated via dopamine D1-like receptors (D1 and D5) is critically implicated in normal cognitive and motor function. Impairment of D1-like signaling is observed in neuropsychiatric and neurological disorders such as schizophrenia, Parkinson's disease and addictive behavior. Therefore, small molecules targeting D1- like receptors are potentially good candidates for treatment of cognitive symptoms in these debilitating disorders. As selective compounds for the functional analysis of D1 and D5 receptor using neurophysiological techniques are still sparse, complete knowledge about their subcellular localization obtained by immunoelectron microscopy may pinpoint a potential differential functional role that these receptors play in the modulation of neuronal circuits in the cerebral cortex, hippocampus and subcortical structures.
Methods: We analyzed the localization of dopamine D5 receptor in the rhesus monkey prefrontal cortex (area 9) using immunohistochemistry at the electron microscopic (EM) level and a subtype specific antibody against D5 receptor.
Results: In addition to the localization of D5 receptor in cell bodies and dendritic shafts, as reported previously (Bergson et al. J. Neurosci. 15: 7821, 1995; Paspalas and Golman-Rakic J. Neurosci. 24: 5292, 2004), a significant number of D5 receptors in layers III and V was observed in axons (28% of total number of axons counted) and dendritic spines (33%). D5 positive axons formed predominantly asymmetric synapses on dendritic spines (83%) and less with dendritic shafts (17%), while D5 positive spines always received asymmetric input. A number of dendrites receiving D5 asymmetric input contained several closely spaced asymmetric inputs, which is clearly a morphological characteristic of interneuronal (GABAergic) dendrites.
Discussion: Association of D5 receptors with axons and spines forming asymmetric synapses suggests the involvement of D5 receptor in the modulation of excitatory, presumably glutamatergic, neurotransmission at the pre- and postsynaptic levels. Present data show that in comparison to D1 receptor (Paspalas and Goldman-Rakic J. Neurosci. 25: 1260, 2005), D5 receptor is more abundant in the presynaptic domain (13% versus 28% of excitatory like synaptic terminals). In addition, since postsynaptic elements of D5 positive axons form asymmetric synapses both with dendritic spines of pyramidal and dendrites of GABAergic neurons, our results also suggest that in the non-human primate prefrontal cortex D5 receptors are involved in the modulation of the excitatory input both to projection and local circuit neurons in the supra- and infragranular layers. Pre- and postsynaptic localization of D5 receptor associated with glutamatergic and GABAergic neurotransmission shows that compounds selectively targeting this receptor may have potential to attenuate cognitive symptoms linked to the dysfunction of the prefrontal cortex in psychiatric disorders such as schizophrenia.

114. Steady State Concentrations After Standard and Higher Doses of Oral Olanzapine in Acutely Ill Patients with Schizophrenia or Schizoaffective Disorder with Suboptimal Prior Response

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Sponsor: Gary Tollefson

Background: An objective of this study was to assess the pharmacokinetic characteristics of standard (10mg and 20mg) and higher(40mg)daily oral doses of olanzapine in acutely ill, non-treatment-resistant patients with schizophrenia or schizoaffective disorder with suboptimal response to current treatment.
Methods: This randomized, double-blind, 8-week, fixed-dose study compared olanzapine treatment with 10 mg/d (n=199), 20 mg/d (n=200) and 40 mg/d(n=200) for patients with schizophrenia or schizoaffective disorder, with baseline BPRS score ≥45, scores on at least 2 of the 4 BPRS positive symptom items ≥4 (moderate), CGI-Severity score ≥4, and less than optimal response to current treatment in the opinion of the investigator. Patients with history of antipsychotic treatment resistance, in particular to atypical antipsychotics, were excluded. The primary outcome measure was a change in Positive and Negative Syndrome Scale (PANSS) total score. Steady-state olanzapine plasma concentrations were determined for a subset of patients (10 mg/d, n= 133; 20 mg/d, n=125; 40 mg/d, n=122) from blood samples collected after daily administration of fixed doses for 2 and 6 weeks. Correlations between olanzapine plasma concentration and PANSS total score, change from baseline in PANSS total score, body weight, and change from baseline in body weight were examined.
Results: The proportion of patients completing the study and time to treatment discontinuation did not significantly differ between dose groups. All 3 dose groups showed statistically significant improvement in PANSS total scores from baseline to endpoint, without significant dose-response relationship (p=.295). Steady-state olanzapine concentrations at 2 and 6 weeks did not differ and were pooled for analysis. Median steady-state olanzapine plasma concentrations of 17, 34, and 69 ng/mL were proportional to doses of 10, 20, and 40 mg respectively. The 10th to 90th percentiles for the concentration distributions of the 10 mg (8 to 35 ng/ml) and 20 mg (12 to 66 ng/mL) dose groups, were contained within the 10th to 90th percentiles for the historical concentration range (5 to 69 ng/mL) for similar doses. Approximately half of the distribution of concentrations for the 40 mg dose group (21 to 140 ng/mL) was above the 90th percentile (69 ng/mL) for a 20 mg dose. Typical covariate effects such as smoking (causing lower concentrations in smokers) and gender (causing higher concentrations in females) were consistently observed in the 10, 20 and 40 mg dose groups as observed historically for olanzapine. There was a lack of correlation between olanzapine plasma concentration and the change from baseline in PANSS total score for responders or non-responders. Although there was a significant difference between the 10 and 40 mg dose groups (p=.002) for mean change in weight (increasing 1.9 kg [10 mg/day] versus 3.0 kg [40 mg/day]), again there was a lack of correlation between the olanzapine plasma concentration and body weight or change from baseline weight. Furthermore, the correlation did not substantially improve when evaluated in sub-groups of obese (BMI >28) and non-obese (BMI < 28) patients.
Discussion: Over 8 weeks, acutely-ill, non-treatment-resistant patients with schizophrenia or schizoaffective disorder responded to all 3 doses of oral olanzapine, without a statistically significant dose-response relationship. Steady-state plasma olanzapine concentrations

were proportional to doses up to 40 mg and typical covariate effects were observed. There were no correlations between olanzapine plasma concentration and change in PANSS total score or body weight.

115. Effects of Paliperidone ER in Patients with Schizophrenia Previously Treated with Risperidone

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Sponsor: George Simpson

Background: This post-hoc analysis assessed the effects of an investigational psychotropic, paliperidone extended-release (ER) tablets, in patients with acute symptoms of schizophrenia who received prior treatment with risperidone.
Methods: Pooled data from over 1600 patients in the intent-to-treat (ITT) population of 3 similar 6-week, double-blind, parallel-group, placebo-controlled trials were evaluated. Patients included in this exploratory analysis were randomized to fixed doses of paliperidone ER 3-12 mg/day or placebo and had received treatment with oral risperidone within 2 weeks prior to randomization. Assessments included the Positive and Negative Syndrome Scale (PANSS), the Personal and Social Performance (PSP) scale, the Clinical Global Impressions (CGI) scale, and adverse event reports. An analysis of covariance model was used to compare between-treatment differences for continuous variables. There was no adjustment for multiplicity.
Results: The primary analysis of pooled data compared paliperidone ER (3-12 mg combined arms) to placebo. A total of 285 patients (paliperidone ER 3-12 mg/day, n=207; placebo, n=78) met the inclusion criteria. In the active treatment group, patients had a mean±SD age of 37.5±11.5 years and a mean±SD length of illness of 11.2±9.8 years. The mean±SD baseline total PANSS score was 92.7±12.2. Patient characteristics were similar in the placebo group and in the overall pooled ITT population. The median duration of prior risperidone treatment was 95 days in the paliperidone ER group and 104 days in the placebo group. The median risperidone dose in both groups was 4.0 mg/day. The study completion rate was 59.9% for paliperidone ER vs 41.0% for placebo. At endpoint, significant improvement was observed in mean±SD PANSS total score (paliperidone ER, –15.1±19.9; placebo, –4.7±23.5 [P<0.001]). There was a significantly greater improvement with paliperidone ER than placebo in all mean PANSS factor change scores (negative symptoms, positive symptoms, anxiety/depression, disorganized thoughts, and uncontrolled hostility/excitement) at endpoint (P≤0.01). Mean±SD PSP scores improved significantly at endpoint for paliperidone ER vs placebo (+8.0±14.2; –2.2±16.1 [P<0.001]). Mean±SD CGI score also improved significantly at endpoint for paliperidone ER vs placebo (–0.8±1.1; –0.2±1.2 [P<0.001]). There was no significant difference in mean±SD SAS change scores at endpoint (paliperidone ER, 0.01±0.2; placebo, –0.05±0.2 [P=0.226]). Adverse events noted in ≥10% of patients were (paliperidone ER vs placebo): headache (13.0% vs 14.1%), agitation (6.8% vs 11.5%), insomnia (11.6% vs 15.4%), and anxiety (6.3% vs 11.5%).
Discussion: Results suggest that in patients with acute symptoms of schizophrenia who received prior treatment with risperidone, paliperidone ER was significantly superior to placebo for improving acute symptoms and functioning, with no unexpected tolerability findings.

116. ACP104 Initial Phase II Results: Putative Antipsychotic Drug and Cognition Enhancer

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Background: ACP-104 (N-desmethyl clozapine) is a new drug with a receptor profile similar to a second generation antipsychotic but demonstrating selective M1/M5 muscarinic agonist activity. This profile suggests that the drug will show two favorable properties in schizophrenia: antipsychotic effects and cognition enhancement. Initial Phase II studies have shown good safety and tolerability and have indicated a drug action that is consistent with the a priori predictions.
Methods: Single rising dose safety studies were carried out across a dose range of 25mg-250 mg orally in 24 volunteers with schizophrenia. Multiple dose studies were conducted in 40 patients over 14 days across a dose range of 25 mg-400 mg bid. Usual measure of safety, tolerability and pharmacokinetics were carried out in both studies. Occupancy at the serotonin-2a receptor was done using PET with 11-C-MDL100907.
Results: Single dose results: Measures in the single dose study indicated good safety and tolerability at doses up to 250 mg. No maximum tolerated dose was reached. Laboratory measures were within normal limits and no significant QTc prolongation was noted at any dose level tested. Sedation was the most frequent side effect, described as mild to moderate in most subjects. Pharmacokinetic analyses suggest an elimination half life of 12-15 hours. Reductions in the total PANSS score were evident even after single doses. Multiple Dose Study: 300 mg bid was identified as the maximum tolerated dose in this escalation regimen. The most frequent adverse events included sedation, increased salivation, constipation and tachycardia. There were 2 SAEs in the repeat dose study: one instance of seizure and one instance of short-lasting fever of unknown origin followed by mild transient leucopenia. In each case, laboratory finding were unremarkable including ECG parameters. Pharmacokinetic analyses from the repeat dose studies suggest that the steady state is established after 6 to 7 days of dosing with an estimated half life of 15-35 hours; the mean steady state trough levels at 200 mg bid are 362 ng/ml. An antipsychotic effect was apparent with subchronic dosing of 200 mg bid and higher. Occupancy study: Drug occupancy at the serotonin-2a receptor at 150 mg was found to be approximately 67% using 11-C MDL100907.
Discussion: These early results indicate that ACP104 is safe and well tolerated across the dose range producing relevant receptor occupancy. Preliminary evidence of antipsychotic activity in psychosis is shown, consistent with its receptor profile. ACP104 will be examined in separate protocols for its antipsychotic efficacy and its cognition enhancing effects. (These studies were sponsored by Acadia Pharmaceutical Co with funding from the Stanley Medical Research Institute and conducted at UTSW, Synergy Clinical Research, Columbia University and JHH)

117. TBPB is a Highly Selective M1 Allosteric Mscarinic Receptor Agonist *in vitro* and Produces Robust Antipsychotic-Like Effects *in vivo*

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Sponsor: Ariel Y. Deutch

Background: Growing evidence suggests that selective agonists of the M1 muscarinic receptor may reduce psychotic symptoms in patients suffering from various neurodegenerative and psychiatric disorders. Previous studies have reported that xanomeline, a M1/M4-preferring muscarinic agonist, significantly reduces psychotic behaviors, including hallucinations and delusions, in both Alzheimer's disease and schizophrenic patients. More recently, N-desmethylclozapine, a major metabolite of the atypical antipsychotic clozapine, has been shown to preferentially bind to M1 muscarinic receptors with an IC50 of 55 nM and to be a more potent partial agonist (EC50, 115 nM and 50% of acetylcholine response) at this receptor than clozapine itself. In addition, pharmacological and site-directed mutagenesis studies suggest that N-desmethylclozapine preferentially activates M1 receptors by interacting with a site that does not fully overlap with the acetylcholine orthosteric binding site. Previous attempts to develop

selective M1 orthosteric agonists have failed in clinical development due to a lack of true specificity for M1 and therefore inducing adverse effects through activation of other muscarinic receptor subtypes.
Methods: In the present study, we have pharmacologically characterized TBPB (1-[1'-(2-Tolyl)-1,4'-bipiperidin-4-yl]-1,3,-dihydro-2H-benzimidazol-2-one), a recently described novel M1-selective agonist *in vitro* using recombinant systems and *in vivo* in several models predictive of antipsychotic activity, including changes in Fos expression and reversal of amphetamine-induced hyperactivity in rats.
Results: In recombinant systems, TBPB robustly increased intracellular calcium in cells expressing M1 receptors (EC50 of 140nM). Moreover, at concentrations up to 10μM, TBPB was without effect at any of the other muscarinic subtypes (M2-M5). TBPB also activated the mutant M1 receptor (Y381A), which is insensitive to orthosteric agonists such as carbachol with a potency similar to that observed at wildtype M1, suggesting that this compound is not acting at a site identical to the orthosteric binding site. Thus, TBPB is a highly selective M1 muscarinic agonist *in vitro*, with properties consistent with those expected for an allosteric agonist. *In vivo*, TBPB dose-dependently reversed amphetamine-induced hyperlocomotion in rats, with effects similar in magnitude to those seen after haloperidol or clozapine treatment. In addition, TBPB induced the expression of Fos in the prefrontal cortex and nucleus accumbens, and thus mimics the actions of xanomeline and clinically-used atypical antipsychotics. However, TBPB did not produce catalepsy at any time point up to 4 hours after drug administration at doses that reversed amphetamine hyperactivity. In contrast, at doses that reverse the effects of amphetamine, haloperidol challenge induced catalepsy. TBPB also did not produce the M2/M3 receptor-mediated effects of salivation, lacrimation, or diarrhea at doses, which reversed amphetamine hyperactivity.
Discussion: Our findings strongly support the hypothesis that selective M1 allosteric agonists mimic many of the antipsychotic-like actions of xanomeline and antipsychotic drugs that predict clinical efficacy, and may be useful in the treatment of psychotic symptoms in patients with neurodegenerative and psychiatric disorders.

118. Clozapine Markedly Elevates Pregnenolone in Rat Hippocampus, Cerebral Cortex, and Serum: Candidate Mechanism for Superior Efficacy?

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Background: Clozapine demonstrates superior efficacy in patients with refractory schizophrenia, but the precise mechanisms contributing to this distinct clinical advantage remain incompletely characterized. Clozapine is also FDA-approved for the treatment of suicidal behaviors in patients with schizophrenia and schizoaffective disorder. Clozapine and olanzapine increase the GABAergic neurosteroid allopregnanolone (Marx et al 2000, 2003; Barbaccia et al 2001), and it has been hypothesized that neurosteroid induction may contribute to the therapeutic actions of these agents. Pregnenolone administration improves learning and memory in rodent models (Flood et al 1992), and decreases in this neurosteroid have been associated with depressive symptoms in humans (George et al 1994). Pregnenolone also enhances neurite outgrowth (Fontaine-Lenoir et al 2006) and impacts microtubule assembly (Murakami et al 2000). These pregnenolone characteristics may be relevant to antipsychotic mechanisms of action. Furthermore, we recently determined that pregnenolone levels in parietal cortex in patients with schizophrenia who died by suicide are significantly reduced compared to pregnenolone levels in patients with schizophrenia who died of other causes (Marx et al 2006). Clozapine-induced pregnenolone elevations could thus potentially contribute to its therapeutic actions on suicidal behaviors. We therefore investigated possible pregnenolone alterations in rat hippocampus and cerebral cortex following clozapine, olanzapine, and other second generation agents as a candidate neurosteroid mechanism contributing to antipsychotic efficacy.
Methods: Antipsychotic agents were studied at doses producing D2 receptor occupancies consistent with clinically comparable ranges, and also consistent with effective doses utilized in numerous rodent models of psychosis. In the first set of experiments, intact, adrenalectomized, and sham-operated male rats received vehicle or clozapine (20 mg/kg) IP, n=6-10 rats per condition. In the second set of experiments, male rats received vehicle, olanzapine (5 mg/kg), quetiapine (20 mg/kg), ziprasidone (10 mg/kg) or aripiprazole (5 mg/kg) IP, n=9 rats per condition. Pregnenolone levels were determined by gas chromatography/mass spectrometry preceded by high performance liquid chromatography purification.
Results: Clozapine markedly elevates pregnenolone in rat hippocampus (13-fold), cerebral cortex (26-fold), and serum (34-fold). Pregnenolone levels in hippocampus are strongly correlated with pregnenolone levels serum ($r=0.987$, $p<0.0001$). Adrenalectomy prevents clozapine-induced elevations in hippocampal and serum pregnenolone levels. Olanzapine also elevates pregnenolone levels, but to a considerably lesser degree compared to clozapine. Quetiapine, ziprasidone, and aripiprazole do not significantly alter hippocampal or cerebral cortical pregnenolone levels at the doses tested in this investigation.
Discussion: Pregnenolone induction may contribute to the therapeutic actions of clozapine and possibly olanzapine. Given the magnitude of clozapine effects on pregnenolone levels in two brain regions and peripheral serum in these investigations, it is possible that marked pregnenolone induction following clozapine may contribute to mechanisms mediating its superior clinical efficacy. Pregnenolone induction may also be relevant to clozapine's therapeutic actions on suicidal behaviors. Hippocampal and serum pregnenolone levels are strongly correlated. It is therefore possible that peripheral serum pregnenolone levels may reflect central pregnenolone levels, potentially providing an accessible surrogate marker for brain levels of this neurosteroid.

119. Plasma Protein Levels of Low and High Abundance Proteins in Patients with Schizophrenia

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Sponsor: Terry Goldberg

Background: We have investigated plasma levels of low and high abundance proteins in 21 monozygotic twin pairs discordant for schizophrenia using Elisa-based technology.
Methods: Plasma samples were tested in duplicates and assayed at different dilutions depending on plasma concentration.
Results: We analyzed ~ 100 proteins in human plasma and detected differences in protein expression levels between cases and controls for eleven proteins, which we suggest correspond to inflammation-T cell related proteins (4), obesity-drug related effects (3) and bone metabolism-drug effects (4). For the first group, chemokines I-309 ($p= 0.01$), Mip 1β ($p= 0.05$), Mip 3β ($p= 0.01$) and lymphotactin ($p= 0.02$) showed overexpression in schizophrenic twins as compared to unaffected cotwins. Within the second group, leptin ($p= 0.002$), PAI 1-active ($p= 0.007$) and PAI 1-total ($p= 0.02$) showed overexpression in cases. The third group, which included RANK ($p= 0.04$), MMP-8 ($p=0.03$), and cytokine IL-17 ($p= 0.03$) showed overexpression in affected twins whereas HGH ($p= 0.001$) was found to be significantly decreased, as compared to unaffected cotwins.
Discussion: Chemokines I-309, Mip 1β, Mip 3β and lymphotactin direct T cells to sites of inflammation or injury. Increased levels of leptin and PAI-1 in plasma can be observed in obese people following antipsychotic treatment. Cytokine IL-17, RANK, MMP-8 and HGH

have been linked to bone homeostasis/osteoclastosis, also probably due to antipsychotic treatment. Our results support a possible role for inflammation in the etiopathology of schizophrenia and demonstrate secondary treatment effects of antipsychotic medications.

120. Direct Measurements of Adiposity During Divalproex Augmentation of Antipsychotic Treatment in Schizophrenia

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Schizophrenia and its treatment are commonly associated with obesity and increases in adiposity. Studies are underway to compare effects of monotherapy with various antipsychotics on direct measures of adiposity, but schizophrenia patients are commonly treated with polypharmacy in the community. Few studies have described the effects of commonly used polypharmacy strategies on changes in adiposity. Increased adiposity can disturb glucose and lipid metabolism via disturbances in insulin sensitivity, and schizophrenia patients experience an increased prevalence of diabetes mellitus in comparison to the general population. Increased adiposity, plasma glucose and lipids are independent risk factors for cardiovascular disease, and schizophrenia patients experience increased cardiovascular (CV) mortality in comparison to the general population. Adiposity can be directly measured using whole body dual energy x-ray absorptiometry (DEXA) and abdominal magnetic resonance imaging (MRI).
Methods: Subjects included patients with schizophrenia chronically treated with antipsychotic medications that were randomized to three months of augmentation with divalproex or placebo.
Results: Preliminary analyses indicate that divalproex augmentation can result in significant increases in direct and indirect measures of adiposity. Covarying the baseline value of the dependent variable, time X treatment group (placebo or divalproex) effects were observed with BMI ($F[1,14]=10.23$, $p=.006$), DEXA total fat ($F[1,13]=7.32$, $p=.018$), and MRI subcutaneous fat ($F[1,9]=7.98$, $p=.020$).
Discussion: Sensitive techniques such as these can be used to carefully assess effects of polypharmacy that may contribute to disturbances in glucose and lipid metabolism and cardiovascular risk in schizophrenia, allowing patients and clinicians to make informed decisions about the risks and benefits of a given polypharmacy strategy.

121. Crybb2 May Alter Sensorimotor Gating and Schizophrenia-Related Cognitive Domains in Mice and Humans

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Sponsor: Manfred Ackenheil

Background: Beta- and gamma-crystallins are structural proteins of the ocular lens and mutations in the corresponding genes lead to cataracts.
Methods: Since expression of Crybb2 (encoding betaB2-crystallin) in non-ocular tissues has been demonstrated, we studied its role in the murine brain by characterization of a novel Crybb2 mouse cataract mutant line. Based on the obtained behavioural data, we hypothesized that genetic variations in Crybb2 are associated with schizophrenia-related endophenotypes and conducted an association study using 501 schizophrenic patients and 1306 controls.
Results: The mutation was mapped to chromosome 5 and characterized as an A→T substitution at the end of intron 5 of Crybb2. It leads to alternative splicing with a 57-bp insertion in the mRNA and to an additional 19 amino acids in front of the 4th Greek key motif. Beside the lens, we determined βB2-crystallin expression in the olfactory bulb, cerebral cortex, hippocampus, and cerebellum. Expression profiling demonstrated a significant regulation of a few genes in the brains of homozygous mutants, particularly, an increased expression of calpain-3 (Capn3) and thymosin-beta-4 (Tmsb4x). Most interestingly, behavioral studies revealed a deficiency in prepulse inhibition (PPI) indicating altered sensorimotor gating. In humans, PPI impairment has a high predictive value for neuropsychiatric dysfunctions associated with schizophrenia. Moreover, the human chromosomal region harbouring CRYBB2 (22q11.2) is linked to schizophrenia and a schizophrenia-related phenotype of reduced P50 suppression (sensorimotor gating) and altered antisaccade eye movement. We demonstrate here that P50 suppression, antisaccade eye movement and cognitive schizophrenia-related endophenotypes (fluency, learning, memory and speed of processing) but not schizophrenia per se are associated with a particular CRYBB2 haplotype. These novel observations warrant further replication in independent samples.
Discussion: This finding is consistent with the hypothesis that altered expression of CRYBB2 in humans contributes to schizophrenia endophenotypes. Moreover, since beta-B2-crystallin is discussed to have Ca2+-binding properties and Ca2+-dependent enzymes are differentially expressed in the homozygous mutants, it is tempting to speculate that the observed behavioural alterations and association with schizophrenia endophenotypes might be linked to Ca2+-signalling.

122. Pregnenolone Alterations in Parietal Cortex are Associated with Death by Suicide in Patients with Schizophrenia

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Sponsor: Dan Blazer

Background: Schizophrenia is associated with a very high lifetime risk of suicide and suicidal behaviors (Palmer et al 2005). Potential neurobiological substrates of suicidality in patients with schizophrenia and other neuropsychiatric disorders merit further investigation. Clozapine was recently approved by the FDA in the United States for the treatment of suicidal behaviors in patients with schizophrenia or schizoaffective disorder (Meltzer et al 2003), but the precise mechanisms contributing to clozapine effects on suicidality are currently unknown. We recently determined that clozapine markedly elevates the neurosteroid pregnenolone in rodent hippocampus and serum, and hypothesized that pregnenolone induction may contribute to the superior efficacy of this antipsychotic (Marx et al 2006a). Furthermore, pregnenolone levels are similarly elevated in patients with schizophrenia and bipolar disorder in both parietal cortex and posterior cingulate compared to control subjects (Marx et al 2006b), a finding that may represent an adaptive or compensatory response. We therefore hypothesize that pregnenolone levels will be reduced in parietal cortex and posterior cingulate in patients with schizophrenia who died by suicide compared to patients with schizophrenia who died by other causes in this pilot investigation. Since pregnenolone levels were similarly altered in patients with schizophrenia and bipolar disorder in both brain regions (Marx et al 2000b), we also determined if pregnenolone is reduced in patients with schizophrenia and bipolar disorder who died by suicide compared to patients with these diagnoses who died by other causes.
Methods: Postmortem tissue was generously donated by the Stanley Foundation. Pregnenolone levels were determined by gas chromatography/mass spectrometry preceded by high performance liquid chromatography purification. Pregnenolone levels were analyzed nonparametrically by Mann-Whitney U test statistic.

Results: Median pregnenolone levels in parietal cortex were significantly lower in subjects with schizophrenia who committed suicide (19.01 ng/g; n=4) compared to patients with schizophrenia who died of other causes (41.86 ng/g; n=11), p=0.04. Median pregnenolone levels in posterior cingulate were not significantly different in patients with schizophrenia who died by suicide (p=0.21). When patients with schizophrenia and bipolar disorder were combined, median pregnenolone levels were significantly lower in parietal cortex in patients who died by suicide (23.38 ng/g; n=13) compared to patients who died of other causes (52.57 ng/g; n=17), p=0.02, and also tended to be lower in posterior cingulate in patients who committed suicide in this combined group (p=0.06).
Discussion: Pregnenolone levels are significantly reduced in parietal cortex in patients with schizophrenia who died by suicide compared to patients with schizophrenia who died of other causes. Pregnenolone levels are also reduced in parietal cortex (significantly) and posterior cingulate (trend) in patients who died by suicide when subjects with schizophrenia and bipolar disorder are combined. Pregnenolone may be relevant to the neurobiology of suicide in schizophrenia and bipolar disorder.

123. Efficacy and Safety of Bifeprunox in the Treatment of Patients with Acute Exacerbations of Schizophrenia: Results of a Dose-Finding Study

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Background: To evaluate the efficacy and safety of bifeprunox in the treatment of patients with acutely exacerbated schizophrenia.
Methods: A 6-week randomized, placebo-controlled, risperidone-referenced, dose-finding study included 589 randomized patients with acute exacerbations of schizophrenia (DSM-IV-TR). Patients were randomly assigned to once-daily bifeprunox 5 mg (n=115), bifeprunox 10 mg (n=120), bifeprunox 20 mg (n=115), placebo (n=119) or risperidone 6 mg (n=120). Bifeprunox doses were titrated, beginning with a dose of 0.125 mg on day 1 and approximately doubled every day until 5 mg (day 6), 10 mg (day 7) or 20 mg (day 8) were reached, while risperidone treatment was titrated over 3 days. The change in the Positive and Negative Symptom Scale (PANSS) total score, from baseline to endpoint (LOCF), was the primary outcome measure. Secondary efficacy measures included: PANSS positive, PANSS negative, PANSS general psychopathology (GPP) score, PANSS-derived Brief Psychiatric Rating Scale (BPRS) score, Clinical Global Impressions-Severity of Illness (CGI-S), CGI-Improvement (CGI-I) scores, and responder rates. Safety and tolerability evaluations included extrapyramidal symptoms (EPS), weight gain, lipid profile, and serum prolactin. Risperidone was included for assay sensitivity.
Results: Reduction in the PANSS total score for bifeprunox was statistically different (P<0.05) from placebo at week 3 and week 6/endpoint for the 20 mg dose. The positive effect of bifeprunox 20 mg was also observed on the secondary efficacy measures PANSS positive, negative, GPP subscales, BPRS, and responder rates. Risperidone 6 mg was positive at week 6. The most common adverse events (incidence >5% and twice for placebo) included: dyspepsia, nausea, vomiting, and constipation. A dose relationship was not evident for any of the most frequent adverse events. Bifeprunox was associated with decreased prolactin levels, and rates of EPS that were comparable to placebo. In addition, compared to placebo, patients receiving bifeprunox 20 mg experienced statistically significant (P<0.05) weight decrease, and demonstrated statistically significant improvements in non-fasting triglycerides (P<0.005) and total cholesterol (P<0.005).
Discussion: In this study, bifeprunox 20 mg was shown to be effective and safe in the treatment of patients with acutely exacerbated schizophrenia. Bifeprunox may have safety advantages, stemming from a decrease in weight, and improvement in the lipid profile.

124. Premorbid Adjustment and Neuropsychological Performance in a Large Cohort of Schizophrenia and Discordant Siblings

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Sponsor: Allan Mirsky

Background: A number of genes, as well as cognitive and environmental factors that contribute to the development of Schizophrenia have been found. While it is widely accepted that patterns of susceptibility to the disease are familial, the mechanisms involved therein are not well understood. It has been shown that schizophrenic probands and their discordant siblings display cognitive deficits in the same domains (i.e. verbal and working memory, sustained attention), suggesting certain patterns of cognitive decline as endophenotypes of the genetic mechanisms for transmission. Indeed, many probands show a decrease in cognitive ability from adolescence through adulthood. While it has been shown that similar, but less marked decline is manifest in non-affected family members, agreement on this matter has not been attained. Additionally, patients with schizophrenia show poor premorbid social and academic adjustment when compared with controls. However, some cohorts show decline beginning in childhood, while in some cognitive dysfunction is not evinced until early adolescence. Small studies have found the same in non-affected family members.
Methods: The current study investigated 237 schizophrenic probands, 185 siblings free of any schizophrenia spectrum diagnoses, and 225 healthy controls. Measures of Wechsler Adult Intelligence Scale (WAIS), Wide Range Achievement Test (WRAT), Premorbid Adjustment Scale (PAS), Four Factor Index of Social Status (SES) and years of education were obtained for every subject. Only PAS childhood and early adolescence subscores were used for the current analyses, as they were thought to predate onset of psychotic illness. WRAT, WAIS, WAIS-WRAT (a measure of cognitive decline), and PAS scores were the main outcome variables, while family SES, years of education, gender, and age were considered as covariates. All covariates were reduced to categorical variables (5 levels for SES, 4 for years of education, and 3 for age), in order to have enough subjects/cell. The main variables of interest were adjusted to correct for the covariates that were found to be significant using an ANOVA model (gender was the only variable that did not require a correction).
Results: After these adjustments, the three diagnostic groups were significantly different on WAIS (M=107.14 SD=8.49, M=105.77 SD=9.18, M=97.92 SD=10.46 for controls, siblings, and probands respectively), PAS childhood (M=.150 SD=.103, M=.181 SD=.110, M=.233 SD=.152), and PAS early adolescence (M=.170 SD=.095, M=.201 SD=.091, M=.269 SD=.144), but not on premorbid IQ (M=107.60 SD=8.00, M=107.57 SD=7.10, M=106.93 SD=8.52). Siblings were also significantly less well adjusted in childhood and early adolescence than controls. Despite patterns of poor premorbid adjustment, siblings of patients with schizophrenia did not show marked cognitive decline (WAIS-WRAT: -0.501 + 9.01), nor did they appear to show significant current cognitive dysfunction. Finally, when looking across all groups, PAS scores in childhood and early adolescence predicted current cognitive ability as measured by IQ ((r = -.2496, p<.001) and r = -.2758, p<.001, respectively).
Discussion: PAS scores in early adolescence (r = -.2059, p<.001), and to a lesser extent in childhood (r = -.1294, p=.002), predicted measures of cognitive decline, suggesting that the declivity in intellectual function begins sometime early in adolescence. Results suggest that heritable mechanisms affect premorbid adjustment of non-psychotic siblings of probands in childhood and adolescence. This is associated with their general cognitive abilities and, to a much lesser extent, with cognitive decline. This data set can be used to identify, in more detail,

which genes contribute to cognitive decline and to social functioning in childhood.

125. Structural and Functional Abnormalities of the Anterior Cingulate Cortex Contribute to Executive Dysfunction in Schizophrenia

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Sponsor: Judith Ford

Background: To perform well on any challenging task, it is first necessary to free your mind from distractions in order to focus on the task at hand and then to evaluate your performance so that you can learn from errors. These two processes, task preparation and performance evaluation, depend on the anterior cingulate cortex (ACC), an essential component of the neural circuitry involved in flexible, adaptive behavior that has been shown to be functionally and structurally abnormal in schizophrenia.
Methods: We scanned 18 patients with schizophrenia and 15 healthy controls with functional MRI as they performed pseudorandom sequences of prosaccade and antisaccade trials to determine whether the ACC functioned abnormally during task preparation and performance evaluation in schizophrenia. Subjects also underwent diffusion tensor imaging to examine the microstructural integrity of the white matter underlying ACC as indexed by fractional anisotropy.
Results: Patients made more antisaccade errors than controls and showed a trend to perform correct antisaccades more slowly. Early in correct antisaccade trials, patients showed abnormally increased activity in rostral ACC, and following antisaccade errors patients showed decreased activity in both rostral and dorsal ACC. Patients also showed reduced fractional anisotropy in both rostral and dorsal ACC that was associated with increased saccadic latency.
Discussion: We interpret these findings as demonstrating structural and functional abnormalities of the ACC in schizophrenia that contribute to deficient task preparation, slower correct performance, and a failure to optimally evaluate and learn from errors. Specifically, increased activation of the rostral ACC in preparation to respond is associated with increased errors and is thought to represent a failure to optimally allocate processing resources away from a task-irrelevant regions and to task-necessary ones. Deceased rostral and dorsal ACC activation following an error suggests reduced affective and cognitive evaluation of performance. Reduced ACC fractional anisotropy may reflect compromised white matter that leads to slower performance. Because task preparation and performance evaluation are essential to optimal performance across such a broad range of tasks, deficits in these processes may exert a generalized blunting effect on cognitive performance in schizophrenia.

126. One Year Stability of Mismatch Negativity in Schizophrenia Patients

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Background: Schizophrenia patients have widespread deficits ranging from abnormalities in sensory processing to impairments in cognition and daily living. Mismatch Negativity (MMN) is an EEG waveform that is passively elicited by infrequent stimuli that occur during the presentation of more frequent stimuli. Previous studies have demonstrated that patients with schizophrenia have robust MMN deficits that are highly associated with level of daily functioning. The aim of the present study was to assess the 1-year longitudinal stability of MMN in both schizophrenia patients and nonpsychiatric adults.
Methods: Schizophrenia patients (n=77) and nonpsychiatric comparison subjects (n=28) underwent EEG testing at baseline and after 12 months. Stability of MMN at electrode Fz was assessed using intraclass correlations (ICC).
Results: Consistent with previous studies, schizophrenia patients had robust MMN deficits at both the first and second session ($p<0.001$). No evidence of a progression of MMN deficits was observed in this chronic cohort of patients. MMN was highly reliable both for patients (ICC=0.93, $p<0.001$) and nonpsychiatric subjects (ICC=0.90, $p<0.001$).
Discussion: The results of the present study indicate that MMN is extremely stable over a 1 year interval in both schizophrenia patients and nonpsychiatric subjects. This high stability supports the use of MMN across multiple applications including as a biomarker associated with functioning and as an endophenotype in genetic association studies of schizophrenia.

127. Clozapine and a Neurotensin-1 Receptor Agonist Reverse Inherent Cognitive Deficits in Brattleboro Rats

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Background: Our laboratory demonstrated that Brattleboro rats (BRATs) exhibit natural deficits in prepulse inhibition (PPI) similar to those exhibited by schizophrenia patients. More recently, we demonstrated that BRATs exhibit deficits in a social discrimination test, which is an ethologically relevant test of memory and attention pertinent to the cognitive deficits seen in schizophrenia. This makes the BRAT a strong model of sensorimotor gating and cognitive deficits relevant to schizophrenia. We have shown that antipsychotics and neurotensin agonists reverse PPI deficits. In this study, we investigated whether clozapine and a neurotensin agonist that enters the CNS could also reverse social discrimination deficits in BRATs.
Methods: We administered subcutaneously to BRATs either saline, one of several doses of clozapine (0.1, 1.0 10 mg/kg) or PD149163 (0.1, 0.3, 1.0 mg/kg) a selective neurotensin-1 receptor agonist designed to enter the CNS after systemic administration. Immediately after drug injections a juvenile rat was placed in the home cage of test rats for 4 minutes. After a 30 minute period in which test rats were alone in their cage, the previously presented juvenile rat and a novel juvenile were reintroduced for a four minute period. The amount of time the test rat spent investigating the familiar and novel rat was recorded. A group of Long Evans rats were also tested with saline as comparators.
Results: Saline-treated Long Evans rats spent less time exploring preexposed juveniles compared to novel juveniles indicating intact social discrimination. In contrast, saline-treated BRATs did not display social discrimination. Clozapine and PD149163 restored social discrimination in these rats.
Discussion: These results provide further support for BRATs as a genetic animal model with strong relevance to schizophrenia and strong utility for antipsychotic drug development. These results also add to the existing evidence that neurotensin agonists are good candidates for potential novel antipsychotic drugs and in addition to their general antipsychotic efficacy they may have therapeutic benefit for the cognitive deficits associated with schizophrenia.

128. Oral Administration of ABS201, a Novel Neurotensin-1 Receptor Agonist, Produces Preclinical Antipsychotic-Like Effects

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Background: Neurotensin (NT) is a 13-amino acid neuropeptide that is localized in areas of the brain implicated in psychosis. The C-terminal NT hexapeptide, NT(8-13), retains the biological activity of the complete NT tridecapeptide. Administration of NT or NT(8-13) directly into the brain reveal that it produces positive effects in pre-

clinical models predictive of antipsychotic efficacy. The NTR-1 receptor subtype has been implicated in these antipsychotic-like effects. Therefore, a NTR-1 agonist would likely have therapeutic potential as a novel antipsychotic drug. The poor ability of NT or NT(8-13) to resist peptidase degradation in the GI tract and the blood and to cross the blood-brain barrier efficiently has hampered the development of a NTR-1 agonist as a drug for psychosis. Various strategies of chemically modifying NT(8-13) to make it more resistant to peptidase degradation have been employed to develop several drug candidates. Studies have shown that some of these compounds enter the CNS after systemic injection and produce robust antipsychotic-like effects in animal models. However, none of these compounds has demonstrated convincing antipsychotic-like effects after oral administration. ABS201 has selective and strong affinity for NTR-1 (Ki=13 nM). It was generated through substitution of a proprietary (Argolyn Bioscience) non-natural α-methylhomolysine for Arg(8) and substitution of t-leucine for iLeu(12) in the NT(8-13) fragment. This modification makes the major proteolytic sites of NT(8-13) resistant to degradation. We investigated its effects after oral administration in a number of animal models predictive for antipsychotic efficacy.
Methods: In one study various does of ABS201 or saline were administered orally by gavage in Sprague Dawley rats and after 60 minutes rats also received amphetamine (0.5 mg/kg) or saline by subcutaneous (SC) injection. Rats were then placed in cages equipped to measure locomotor activity by infra-red beams. In separate studies various doses of ABS201 or saline were administered orally to Brattleboro rats, or to Brown Norway rats, two strains that have natural schizophrenia-like deficits in prepulse inhibition (PPI). After 60 minutes PPI was measured.
Results: Orally administered ABS201 significantly antagonized amphetamine-induced locomotor activity. It also significantly increased PPI in Brattleboro and Brown Norway rats.
Discussion: Orally administered ABS201 exhibited antipsychotic-like effects in each of the preclinical paradigms studied. This is the first demonstration of a neurotensin agonist producing antipsychotic-like effects after oral administration and as such represents a significant milestone in the effort to develop a novel antipsychotic drug that targets central NTR-1 receptors. Reversal of amphetamine-induced hyperlocomotion is a classic test of antipsychosis, and demonstrates that ABS201 can inhibit mesolimbic dopamine transmission. Brattleboro rats and Brown Norway rats have inherent PPI deficits, similar to schizophrenia patients. PPI deficits in Brattleboro rats are ameliorated by acute administration of atypical but not typical antipsychotics, similar to findings in schizophrenia patients. PPI deficits in Brown Norway rats have been shown to be resistant to typical and atypical antipsychotics, therefore these animals may be a model of antipsychotic drug resistance, a common phenomenon in schizophrenia. The results suggests that ABS201 is an auspicious candidate for further development as a potential novel antipsychotic that may exhibit robust efficacy for symptoms of schizophrenia.

129. Remission in Schizophrenia: A Comparison of the Cost-Effectiveness of 2 Dose Regimens of Ziprasidone Versus Haloperidol Treatment in a 3-Year Double-Blind Extension Study

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Sponsor: Richard Shader

Background: Long-term cost-effectiveness in the treatment of schizophrenia has not been well studied for second-generation (atypical) antipsychotics, despite questions from US managed care and other payers, on their value in comparison with conventional agents. We compared the cost effectiveness of 2 dose regimens of ziprasidone (80-160 mg/d given BID, n = 72; 80-120 mg/d given QD, n = 67) and haloperidol (5-20 mg/d, n = 47) in terms of cost per patient achieving full remission according to consensus-based operational criteria.
Methods: Data used in this analysis were collected in a 40-week core trial and 156-week continuation study. One hundred and eighty six subjects completed the 40-week core phase and entered the 3-year double blind extension study. Efficacy variables included remission rate, defined according to recently proposed criteria[1] and Quality of Life Scale (QLS) scores, and were analyzed over time using generalized estimating equations. A resource utilization questionnaire was administered to obtain data on direct medical costs, social services, criminal justice, and caregiver out-of-pocket costs. Costs were assessed from a payer perspective were discounted over four years.
Results: Thirty-seven percent of subjects completed the full 3-year continuation phase. Ziprasidone treatment was associated with a significantly greater likelihood of achieving full remission in the 6 months preceding the last visit (Week 196 or early termination; $P < .05$). Longitudinal assessment of cross-sectional remission and QLS scores in the continuation phase demonstrated superior improvement (slope or trends) for QD and BID ziprasidone regimens compared with haloperidol. Results of the cost analyses will be presented in terms of cost per patient achieving remission and the cost per quality adjusted life year gained.
Discussion: In this double-blind, long-term study, ziprasidone was associated with continued improvement in remission rate and quality of life, in contrast to haloperidol. The results of this study will address the long-term cost-effectiveness of ziprasidone compared with haloperidol in the treatment of schizophrenia. Reference: 1. Andreasen NC, Carpenter WT Jr, Kane JM, Lasser RA, Marder SR, Weinberger DR. Remission in schizophrenia: proposed criteria and rationale for consensus. *Am J Psychiatry.* 2005;162:441-449.

130. Clozapine Underutilization and Discontinuation in African-Americans Due to Leucopenia

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Sponsor: Robert R. Conley

Background: Clozapine is the only antipsychotic medication approved for treatment-resistant schizophrenia and its superiority compared to conventional antipsychotics in treatment-resistant schizophrenia is well established. Additionally, recent findings from the CATIE II effectiveness trial demonstrate the superiority of clozapine over other second-generation antipsychotics in a population of patients who prospectively failed an optimized antipsychotic trial. Clozapine, however, is underutilized in the US likely due in part to its frequent monitoring and serious side effects. Moreover, racial disparities in the use of clozapine have been observed, with African-Americans less likely to receive this medication than Caucasians. The superior effectiveness of clozapine relative to other antipsychotics merits increased efforts to encourage greater use in appropriate patients and to more efficiently monitor for side effects. A hypothesized explanation for the disparity in clozapine prescribing may be related to the requirements for white blood cell (WBC) monitoring. African-Americans generally have lower than normal WBC values known as benign ethnic neutropenia. It has been estimated that approximately 20% of African-Americans may be deemed ineligible for clozapine due low normal values. Further, up to one quarter of African-American patients may have clozapine discontinued for this reason. Recent publications have challenged current prescribing guidelines of clozapine in African-Americans. Thus, the aim of the current study was to compare the rates of clozapine discontinuation and reasons for discontinuation including leucopenia or agranulocytosis, in a large population of African American and Caucasian patients.
Methods: All data in this study were derived from the Clozapine Authorization and Monitoring Program (CAMP) database from the

State of Maryland. Between 1/1/89 and 12/31/99 and 1,875 patients with treatment resistant schizophrenia were treated with clozapine.
Results: During this period, 5.3% (31/588) of African-Americans and 2.4% (31/1287) of Caucasians discontinued clozapine treatment due to leucopenia (Chi-Square=10.35, df=1, p=0.001). No African-American patients developed agranulocytosis while eight Caucasian patients (0.62%) developed this blood dyscrasia. Discontinuations due to leucopenia occurred throughout treatment. Discontinuations due to agranulocytosis occurred primarily in the first 18 weeks (7/8; 87.5% patients with agranulocytosis).
Discussion: It is likely that African Americans had clozapine discontinued unnecessarily due to benign ethnic neutropenia. We concur with recent recommendations to acknowledge differences in WBC values in African-Americans and to modify prescribing guidelines or formally acknowledge benign ethnic leucopenia like in other countries in order to facilitate greater use of clozapine in these patients.

131. Family History of Affective Illness in Schizophrenia Patients and Cognitive Outcomes

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Sponsor: Travel Awardee, NIMH, 2006

Background: Schizophrenia is a heterogeneous disorder with respect to cognitive phenotypes and treatment response. Recent studies suggest better baseline cognitive capacity in schizophrenia is related to better outcomes and better treatment response. This evidence has prompted strong treatment efforts to improve cognition in people with schizophrenia. However, the association between cognition and treatment response could be due to their independent association with another latent variable, namely a specific etiology. This would suggest that schizophrenia cases with better cognition may have a different subtype of schizophrenia that happens to also be more treatment responsive. Given that affective psychoses are associated with better outcomes, we examined if having a family history of affective disorder was related to cognition in schizophrenia patients.
Methods: Family history was rigorously determined for 398 patients with schizophrenia spectrum disorders admitted to an inpatient research unit. At least one family informant provided information about all 1st and 2nd degree family members. Extensive cognitive assessments were conducted on a subsample of these patients by researchers blind to patient information. The neuropsychological battery included overall measures of intellectual functioning (i.e., WAIS-R), specific neuropsychological measures of executive functioning (i.e., verbal fluency, speed of processing, cognitive flexibility), and memory. Mean scores on neurocognitive measures were analyzed across family history groups covarying for relevant demographic factors.
Results: We found patients with a family history of affective illness scored significantly higher on Full Scale IQ scores ($F(3, 152) = 5.35$, $p<.01$), Performance IQ scores ($F(3, 152) = 3.51$, $p<.05$), and Verbal IQ scores ($F(3, 152) = 7.07$, $p<.001$). Patients with a family history of schizophrenia scored the lowest. We found a similar pattern of performance on specific neuropsychological measures of executive functioning. In particular, patients with a family history of affective illness demonstrated significantly better speed of processing ($F(3, 156) = 5.04$, $p<.01$) and cognitive flexibility ($F(3, 156) = 5.79$, $p<.01$) on a visual-motor task (i.e., Trails A and B). Surprisingly, patients with no family history of schizophrenia or affective disorder performed the worst on these tasks. Patients with a family history of affective illness scored significantly higher on a verbal fluency task (i.e., FAS) ($F(3, 161) = 3.69$, $p<.05$), and a verbal task of cognitive flexibility (i.e., Stroop) ($F(3, 143) = 5.15$, $p<.01$). In terms of the Wisconsin Card Sorting Task, we found no significant differences between the family history groups in problem solving and abstraction performance. However, female patients without a family history of either illness made the most perseverative errors on this task, and the males in this family history group made the least amount of errors. There were no significant differences between the family history groups on overall memory performance.
Discussion: Our findings suggest that a family history of affective disorder is associated with better cognition in schizophrenia. This suggests some cases that clinically are indistinguishable from schizophrenia may have a presumptive etiology that makes them biologically more similar to affective disorder, which may explain their better treatment response.

132. Predictors of Outcome after 4 Months of Treatment for a First Episode of Schizophrenia

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Sponsor: Arthur Rifkin

Background: We examined predictors of outcome after 4 months of treatment for a first episode of schizophrenia.
Methods: One hundred twelve subjects (70% male; mean age 23.3 (SD = 5.0) years) with first-episode schizophrenia (75%), schizophreniform disorder (17%) or schizoaffective disorder (8%) were randomly assigned to treatment with olanzapine (2.5 to 20 mg daily) or risperidone (1 to 6 mg daily). Response criteria required ratings on two consecutive visits of mild or better on the SADS-C+PD items severity of delusions, severity of hallucinations, impaired understandability, derailment, illogical thinking, bizarre behavior plus a rating of very much improved or much improved on the CGI. We also examined the stability of response among subjects who met response criteria. Subjects were classified as failing to maintain response during the acute treatment phase if they no longer met criteria for substantial improvement at any time after the two visits that established their treatment responder status. Variables examined as potential predictors of initial response and response stability were: sex, social class, premorbid social functioning, duration of untreated psychiatric symptoms, duration of untreated psychosis (DUP), severity of positive, negative and depressive symptoms at study entry, alcohol, marijuana or other psychoactive substance use (assessed separately for before study entry and for during study treatment) and motor side effects during treatment (Parkinsonism, EPS, akathisia).
Results: 49.1% (95% CI: 38.7%, 59.6%) of patients met response criteria and among those 29.9% (95% CI: 13.5%, 46.2%) failed to maintain their response. In univariate analyses, the following variables were significantly ($p<0.05$) associated with less likelihood of response to treatment: poor premorbid social functioning, longer DUP, alcohol use before study entry and the development of EPS during treatment. In a multivariate analysis including sex and medication assignment as factors, alcohol use ($p<0.01$) and the development of EPS ($p<0.02$) remained significant predictors of treatment response but DUP ($p<0.06$) and premorbid functioning ($p<0.29$) did not. In univariate analyses, both alcohol use and marijuana use during treatment were significantly ($p<0.05$) associated with failure to maintain response; poor premorbid social functioning was associated with failure to maintain response but the difference did not reach statistical significance ($p<0.07$). Alcohol and marijuana use during treatment were highly correlated, thus we used a composite measure for our multivariate model. In this model that included sex and medication assignment as factors, both the composite substance use measure ($p<0.04$) and poor premorbid social functioning ($p<0.03$) were significant predictors of failure to maintain response.
Discussion: Only about half of our newly treated patients met criteria for response. Our finding that substance use occurring either before or during the trial affected outcome emphasizes the consistently reported negative impact of substance use on early treatment outcome of first episode schizophrenia. Our finding that poor premorbid social adjustment is associated with short-term response instability complements data from a previous study that poor premorbid social adjustment is associated with higher relapse risk over the first 5 years of illness.

133. Incidence and Severity of Tardive Dyskinesia in Patients Receiving Aripiprazole or Haloperidol for the Treatment of Schizophrenia or Schizoaffective Disorder

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Sponsor: William H. Coryell

Background: Tardive dyskinesia (TD) is a debilitating and often persistent movement disorder associated with long-term antipsychotic treatment. Clinical trials suggest that patients receiving atypical antipsychotic medications have a lower propensity to develop movement disorders than those receiving conventional agents. Aripiprazole is a novel antipsychotic with a unique mechanism of action, functioning as a partial agonist at the dopamine D2 and serotonin 5-HT1A receptors agonist, and as an antagonist at the serotonin 5-HT2A receptor. Because dopamine D2 receptor antagonism is thought to be a contributor to antipsychotic-induced TD, we sought to determine the incidence of treatment-emergent TD during the long-term treatment of schizophrenia or schizoaffective disorder with aripiprazole or haloperidol.
Methods: We conducted a post hoc analysis of pooled data collected from two 52-week double-blind trials involving 1294 patients treated either with aripiprazole 20-30mg/d (N=861) or haloperidol 5-10mg/d (N=433). Treatment-emergent TD was identified based on Research Diagnostic Criteria (RDC) extracted from the Abnormal Involuntary Movement Scale (AIMS) (Schooler-Kane criteria). According to these criteria, TD is defined as a score of 1 (mild) in two or more body regions (AIMS items 1-7), OR a score of 2 (moderate) or higher in one. Severity of RDC-defined treatment-emergent TD was extracted from the AIMS severity item.
Results: In patients without baseline TD (N = 1177), the rate of new-onset TD at any time point following randomization was 5.09% for aripiprazole-treated patients, compared with a rate of 11.76% for haloperidol-treated patients ($P < 0.0001$). Using a stricter definition of RDC-defined TD on the last two study visits, the rates of new-onset TD were 0.25% in aripiprazole-treated patients versus 4.09% in haloperidol-treated patients ($P < 0.0001$). In the stricter analysis, the severity of new-onset TD was mild in 100% of aripiprazole-treated patients. In haloperidol-treated patients, new-onset TD was 68.75% mild and 31.25% moderate or severe. The mean baseline to endpoint increase in AIMS score was significantly greater in haloperidol- versus aripiprazole-treated patients in both LOCF (N=1177, $P = 0.0001$) and OC (N=427, $P < 0.0001$) analyses.
Discussion: Our findings indicate that treatment with aripiprazole is associated with a significantly reduced risk of new-onset tardive dyskinesia compared with haloperidol in patients with schizophrenia or schizoaffective disorder treated for up to 52 weeks. Aripiprazole's dopamine D2 partial agonist and/or serotonin 5HT2A antagonist receptor binding profile may contribute to its favorable safety profile with respect to treatment-emergent TD.

134. Differential Effects of Various Antipsychotics on Plasma Glucose and Insulin Levels in the Mouse

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Sponsor: Lisa H. Gold

Background: Some, but not all, atypical antipsychotic treatments have been associated with weight gain, hyperglycemia, lipid abnormalities and the development of type II diabetes in patients(1). Previous studies indicate that acute administration of antipsychotic drugs can induce a marked increase in plasma glucose levels in mice(2,3). The aim of the present studies was to characterize the acute effects of various typical and atypical antipsychotics on plasma glucose and insulin levels in mice. For selected compounds, plasma free drug concentrations were also determined to relate findings to clinically relevant drug exposures.
Methods: Male mice received a single, i.p. injection of an antipsychotic drug or vehicle. Blood samples were collected via retro-orbital bleeding at 1h or 3h post-dose. Plasma glucose and insulin were measured by enzymatic and ELISA methods, respectively. In preliminary studies, comparing 3 mouse strains (CD-1, C57BL/6, FVB/N), we found that the FVB/N mice were the most sensitive to the hyperglycemic effect of clozapine, thus this strain was used in all subsequent experiments. Dose-response studies were conducted with clozapine, olanzapine and ziprasidone, including measurements of free plasma drug concentrations. In order to probe the mechanism of antipsychotic-induced hyperglycemia, the effects of pretreatment with the ganglionic blocker, hexamethonium, and the α2-adrenergic receptor antagonist, yohimbine, were examined.
Results: Administration of clozapine (20 mg/kg), olanzapine (5 mg/kg), quetiapine (10 mg/kg), perphenazine (10 mg/kg) and chlorpromazine (10 mg/kg) induced significant increases in plasma glucose by 140, 98, 97, 120 and 144% above basal levels, respectively. In contrast, ziprasidone (10 mg/kg), aripiprazole (20 mg/kg), and haloperidol (2 mg/kg) did not significantly alter glucose levels. Risperidone (2 mg/kg) reduced plasma glucose (-30%) and markedly enhanced plasma insulin. None of the other drugs had significant effects on insulin levels. Subsequent dose-response studies (1, 3 and 10 mg/kg) on selected compounds revealed that clozapine and olanzapine induced significant elevation in glucose at doses of 3 and 10 mg/kg. These doses of clozapine and olanzapine yielded plasma free concentrations that were in the range and one order of magnitude higher than therapeutic plasma levels, respectively. In contrast, ziprasidone (1, 3 and 10 mg/kg) did not induce hyperglycemia, even at the highest dose that produced plasma free concentrations that were about 50-fold higher than therapeutic plasma levels. Finally, chlorpromazine- and clozapine-induced hyperglycemia was inhibited by pretreatment with either hexamethonium (50 mg/kg) or yohimbine (5 mg/kg).
Discussion: These data indicate that the FVB/N mouse is a sensitive preclinical strain for studying metabolic effects of antipsychotics. Glycemic effects of antipsychotics in mice may be predictive of clinical liability since drugs that produced marked, acute hyperglycemia in mice have been linked to glucose dysregulation and the development of diabetes in patients. The inhibition of antipsychotic-induced hyperglycemia by hexamethonium and yohimbine suggests this effect is driven by a centrally-mediated activation of the sympathetic autonomic nervous system. 1) American Diabetes Association et al. (2004) Obesity Res 12:362-368. 2) Nakadate T. et al. (1980) Eur J Pharmacol 64:107-113. 3) Dwyer DS & Donohoe D. (2003) Pharmacol Biochem Behav 75:255-260.

135. Genomic Analysis of Neurons from a Subnucleus of the Mediodorsal Thalamus in 3 Psychiatric Diseases

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Sponsor: Emmanuel Landau

Background: The mediodorsal nucleus (MDN) of the thalamus has been reported to have a reduced volume in schizophrenia (SZ) in several postmortem and in vivo studies and reduced neuronal number in several postmortem studies. In addition, PET and MRI studies have revealed a relative reduction of glucose and oxygen utilization, while in situ hybridization studies have suggested abnormalities of dopaminergic and glutamatergic neurotransmission. A functional circuit involving neurons from the parvocellular subregion of the MDN, (MDNp), and the dorsolateral prefrontal cortex (DLPFC) has

been implicated in SZ. The molecular underpinnings of the dysfunction in this neuroanatomical circuit have yet to be defined. It is, therefore, important to examine this circuit for SZ-associated abnormalities of gene expression and to determine whether such disorders are specific to SZ or shared by other mental illnesses. While microarray studies of DLPFC in schizophrenia have been previously reported, similar studies in thalamic subregions have not.
Methods: : Postmortem tissue samples were provided by Stanley Medical Research Institute from subjects with SZ, depression (DE), bipolar mood disorder (BI) and non-psychiatric controls. Accumulatively collected neurons from the MDNp were harvested by laser capture microdissection. Extracted RNA underwent T7-based linear amplification of poly (A)-tailed RNA. Genome-wide microarray analysis of gene expression employed Affymetrix U133 Plus 2.0 GeneChips. A total of 5602 probes were qualified for further statistical analysis after a series of filtering criteria. Genes differentially expressed between normal control and diseased brains were identified with ANOVA controlled by FDR <0.05. Genes demonstrating mean fold change >=1.7 were subjected to subsequent global statistical or functional analysis within or among disease groups. Over-represented functional groups of affected genes were categorized based on Gene Ontology (GO) Consortium classification, KEGG and BIOCARTA pathways as well as manually curated pathways / gene networks by Ingenuity System.
Results: Our microarray findings indicate that the largest number of dysregulated genes were in the schizophrenia group (1,141) vs. depression (656) and bipolar (503) as compared to control. The number of genes unique to each disease state were 762, 326 and 215 (SZ/DE/BI, respectively). One hundred nineteen dysregulated genes were common to all disease states. No significantly differentially expressed genes were found when subjects were grouped by gender or history vs. no history of psychosis. A number of neuron-related genes were identified in one or more disease states (for example: pallidin homolog, Rab 14 (neurotransmitter secretion), vesicle related genes (i.e.: Rab 6C, 7, 21), neurexin 1 and 3, neuronal growth regulator 1). Functional categorization of over-represented differentially expressed genes based on GO and pre-defined metabolic or signaling pathways were identified in each of the disease states, many of which demonstrated uniqueness to a particular disease state. Shared functional gene groups within at least two disease states were also identified, such as proteasome, ubiquitin mediated proteolysis, Wnt signaling.
Discussion: Transcriptome analysis of a thalamic neuronal subpopulation isolated by laser capture microdissection coupled with T-7 linear amplification and microarray as a high throughput screening of the whole genome in three disease states makes it possible to identify cellular processes and functional pathways common to, as well as unique for, various psychiatric disease states.

136. Understanding Metabolic Networks in Atypical Antipsychotic Drug Treatment: A Proteomics Approach

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Background: Microarray studies after drug treatment have contributed to a better understanding of mode of actions of antischizophrenic drugs, both typical and atypical. However, the reported data do not include knowledge on post-translational modifications, protein translocation, enzyme activity, or the networks implicated in drug metabolism, since all this information is not encoded in gene sequences. In this study we present a comparison between the effects of clozapine (an atypical antipsychotic) and a novel neurotensin analog (NT69L) at the proteomic level to determine differences in protein expression following acute drug administration in a rodent model.
Methods: Four male Sprague–Dawley rats (150–250 g) were used per group treatment. Animals in each group were injected intraperitoneally with clozapine (10 mg/kg) or NT69L (1 mg/kg) once (acute treatment), while control animals were injected once with an equal volume of saline vehicle (0.9% NaCl). Each animal was decapitated 1 h after the last drug administration and the brains were dissected on ice. The pre-frontal cortex (PFC) was isolated and frozen at -85 Celsius. Proteins were extracted from the PFC of each animal according to solubility using ReadyPrep Sequential Extraction Kit (Bio-rad) and total protein concentration in each tissue homogenate was measured using the Coomassie Protein Reagent assay (Pierce). 2D gel electrophoresis was performed at the Mayo Proteomics Research Center in Rochester,MN using standard protocols. Gels were silver stained, scanned, and analyzed by PDQuest software (Bio-Rad).
Results: We were able to obtain differential expression profiles of cytosolic proteins from rat PFC after acute drug treatment and we were able to establish a comparison with those of a control group. Although 983, 1120, and 858 cytosolic proteins were expressed in the control (saline-injected), NT69L- and clozapine-treated groups, respectively, only 421 were present in all groups. Analysis of proteins up-regulated by two fold or more upon drug treatment revealed 42 in the clozapine and 52 in the NT69L group, when compared to the saline control. Only 15 of those proteins were found to be up-regulated by two fold or more by both drugs. Statistical analysis of the presence of these spots in four replicate sets of gels revealed 8 spots that were significantly up-regulated in the clozapine and NT69L groups with respect to the control. These proteins were identified and mapped in the gels using PDQuest, reduced with DTT, alkylated with iodoacetamide, digested in situ with trypsin and identified by nanoflow liquid chromatography electrospray tandem mass spectrometry (nano LC-ESI-MS/MS) using a ThermoFinnigan LTQ Orbitrap Hybrid Mass Spectrometer coupled to an Eksigent nano LC-2D HPLC system.
Discussion: Following analysis, a network of possible key pathways implicated in the metabolism of atypical antipsychotic drugs was developed to contribute to the elucidation of the mode of action of novel, potential antipsychotic drugs such as NT69L. (Supported by Mayo Foundation for Medical Education and Research and NIMH grant MH71241).

137. Prepulse Inhibition of Startle Is Reduced in Family Members of Schizophrenia Patients

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Sponsor: Burt Angrist

Background: Patients with schizophrenia have difficulty screening out irrelevant stimuli, and often have the experience of sensory flooding. These "gating deficits" may contribute to the thought disorder, cognitive fragmentation, and hallucinations that are so debilitating to these individuals. The acoustic startle response is a reflex contraction of the skeletal muscles in response to a sudden acoustic stimulus. It occurs across mammalian species, and can be easily measured. The modulation of this reflex by a preliminary nonstartling stimulus is termed prepulse inhibition of acoustic startle (PPI), a paradigm that is used as an operational measure of sensorimotor gating. In consonance with the schizophrenia symptoms that are suggestive of gating deficits, many patients with schizophrenia have deficits in PPI when compared to healthy controls. The brain regions that modulate PPI include the hippocampus and prefrontal cortex, areas that are implicated as being abnormal in schizophrenia. Our prior work and work from other labs suggests that PPI impairment in schizophrenia persists despite treatment and hence may be a trait related abnormality. An endophenotype is a measurable trait or phenotype discoverable by a biological test. Using an endophenotype rather than presence or absence of disease is a powerful tool in the study of diseases with complex polygenic etiologies such as schizophrenia. The goal of the endophenotype approach is to narrow the defined phenotype such

that a more homogeneous genotype is expected, making it much more fruitful and to conduct genetic studies. Based on several lines of evidence, it has been suggested that PPI may represent a heritable endophenotype in schizophrenia.
Methods: We examined acoustic startle and PPI in 85 schizophrenic patients, 41 unaffected first-degree relatives of schizophrenic patients, and 82 healthy controls. We tested acoustic startle on pulse alone trials and in three prepulse + pulse trials with interstimulus intervals of 30, 60 and 120 ms.
Results: A mixed model ANOVA computed on percent PPI did not detect differences between groups in any trial type. We subsequently categorized all subjects into two groups: inhibitors, who exhibited lower startle magnitudes during prepulse + pulse trials compared to pulse alone trials, and non-inhibitors, who had greater startle magnitude on prepulse + pulse trials than on pulse-alone trials. We then used repeated measures logistic regression, solved using generalized estimating equations, to determine if the percentage of inhibitors differed across groups. We found that there were significantly fewer inhibitors in the schizophrenia group (78.1%, $p = 0.009$) and families (82.8%, $p = 0.018$) compared to the control group (84.7%) during the 60 ms prepulse + pulse trials (Chi-square for overall model = 7.25, $df = 2$, $p = 0.03$). There was no significant difference between the schizophrenic and family groups.
Discussion: These results confirm our hypothesis that impairments in PPI are seen not only in schizophrenic patients, but also in their unaffected family members, suggesting that PPI deficits have a genetic component. We are currently extending our investigation with a heritability analysis of PPI in families. If impaired PPI emerges as a heritable endophenotype in schizophrenia, a future direction will be to use PPI to inform genetic studies in this disease.

138. Insight in Patients with First-Episode Schizophrenia

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Sponsor: Thomas Ban

Background: The early phase of psychosis constitutes a critical period for treating this illness. It has major implications for the secondary prevention of impairments and disabilities. It is very important to identify symptoms influencing the outcome. Impaired insight is common in schizophrenia and may be related to poor treatment adherence. Our previous findings concerning acute treatment responsivity showed that in the first psychotic manifestation the most frequently observed symptoms were lack of judgement and insight, which persisted at discharge from the index hospitalization. Aim of the study: To compare insight impairment, including its temporal changes, between remitters and nonremitters in patients with first-episode schizophrenia.
Methods: Males, consecutively hospitalized with diagnosed first-episode schizophrenia (according to ICD 10), who provided written informed consent, and were reassessed at the one-year follow-up were included. The psychopathology was evaluated using the Positive and Negative Syndrome Scale prior to acute treatment – on admission; at the end of the acute treatment – at discharge; at the one-year follow-up. Insight was measured using item G12 from the PANSS.
Results: 93 patients (mean age 23 years, mean duration of illness 0.77 years) were reassessed after one year. 73/93 patients (78%) fulfilled the criteria for remission. When compared, remitters and nonremitters showed no significant difference in impaired judgement and insight on admission. The mean value of this item was significantly lower at discharge even in nonremitters; however, a significantly higher value was found after 1 year in nonremitters. In remitters the impaired insight decreased significantly at discharge and there was a significant additional decrease after one year. In nonremitters there was a significant decrease at discharge; however, a significant increase was observed after one year. A minimum score of 4 in the individual items was required for symptom presence. In the 73 remitters the rate of insight impairment was 79.4% on admission, 46.6% at discharge and 10.9% after one year; the same values were 90%, 20% and 70% in the 20 nonremitters. In both remitters and nonremitters the lack of judgement and insight was the first or second most frequently observed item at all 3 time points. The impaired insight on admission was strongly associated with the overall symptomatology, including positive, negative and general psychopathology on admission in both remitters and nonremitters. Only in remitters was the impaired insight at discharge associated with symptoms at discharge, on admission and also after 1 year. The impaired insight at the 1-year follow-up was associated with some symptoms after one year in both remitters and nonremitters.
Discussion: Insight may be state dependent, especially in the patients with a good outcome. Attitudes towards treatment and insight into the illness may vary during the course of the illness. However, more longitudinal prospective studies are needed to verify such state-related change, and the factors that may underlie the acquisition of insight.

139. Factors Associated with Improved Psychosocial Functioning in Patients with Schizophrenia

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Background: The advent of effective antipsychotic medications has raised expectations that treatment will lead not only to clinical improvement, but also to better functional outcomes. This analysis explored factors associated with improved psychosocial functioning in stable patients with schizophrenia or schizoaffective disorder.
Methods: Post-hoc analysis of data from the intent-to-treat (ITT) population (n=323) of an international, double-blind, 1-year study of risperidone long-acting injectable, 25 or 50 mg every 2 weeks. Regression models were used to identify factors associated with improved psychosocial functioning. Dependent variables were change in scores at endpoint on two clinician-rated measures of function: PSP and LOF. PSP evaluates patients' overall personal and social functioning and is rated on a 0 to 100 scale (increasing values indicate improved functioning and autonomy). LOF is a rating of overall level of functioning, with higher values indicating better functioning. Independent variables included in the model were age, sex, continent, prior antipsychotic dose, long-acting risperidone dose, duration of illness, duration in study, PANSS insight item (G12) and the PANSS negative symptoms factor (items: blunted affect, emotional withdrawal, poor rapport, passive social withdrawal, lack of spontaneity, motor retardation, and active social avoidance). Since PANSS factor scores tend to be highly correlated, a multiple regression model was initially run with these factors and revealed that PANSS negative symptom factor was the most robust predictor of PSP. Therefore, only the PANSS negative symptom factor was included in the final regression model.
Results: The mean (±SD) PSP total score improved significantly, from 62.1(14.2) at baseline to 64.1(14.0) at endpoint ($P=0.003$). A multiple linear regression model found significant factors associated with change in PSP total score were: duration in study (estimate: 0.03; $P<0.001$), indicating a direct numerical relationship, such that each month in the study is associated with a 0.9-point increase in PSP total score; change in PANSS insight score (estimate: –1.73; $P<0.01$), suggesting that a 1-point improvement in insight is associated with a 1.7-point improvement in PSP; and change in PANSS negative factor score (estimate: –0.72; $P<0.001$), each 1-point improvement in negative factor score is associated with a 0.7-point improvement in PSP. Significant factors associated with change in LOF total were: duration in study (estimate: 0.005; $P<0.05$) indicating a direct numerical relationship, such that each month in the study is associated with a 0.15-point increase in LOF total score, and change in PANSS negative factor score (estimate: –0.23; $P<0.0001$), indicating that each 1-point

improvement in negative factor score is associated with a 0.23-point improvement in LOF total. A multivariate regression model using both LOF total and PSP total change scores as dependent variables identified change in insight ($P<0.01$), duration in study ($P<0.001$), and change in PANSS negative factor score ($P<0.0001$) as significantly associated factors. Other variables tested, including dose of long-acting risperidone, did not emerge as significant factors.
Discussion: These data show that distinct factors (change in insight, negative symptoms, and duration in study) may be associated with improved psychosocial functioning in stable patients with schizophrenia or schizoaffective disorder.

140. Changes in Brain Kynurenic Acid Levels Alter the Response of Clozapine on Midbrain Dopamine Neurons
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Sponsor: Sven Ove Ogren

Background: Kynurenic acid (KYNA) is an endogenous glutamate-receptor antagonist, preferentially blocking NMDA-receptors. The compound has been implicated in the pathophysiology of schizophrenia. In the present in vivo electrophysiological study we analyze whether drugs that increase or decrease KYNA formation, respectively, are able to influence the response to the atypical antipsychotic drug clozapine on midbrain dopamine neurons.
Methods: Extracellular single unit recording techniques.
Results: In order to modulate the synthesis of KYNA we used NSAIDs with various selectivity for cyclooxygenase (COX)-1 or COX-2. In control rats intravenously administered clozapine (0.625-10 mg/kg) increased the firing rate and the burst firing activity of midbrain dopamine neurons. Administration of indomethacin (50 mg/kg, i.p., 1-4 h), a COX-inhibitor with a preferential selectivity for COX-1, which produced a significant elevation in brain KYNA levels, reversed the excitatory action of clozapine into an inhibitory response. In contrast, the COX-2 selective inhibitors parecoxib (25 mg/kg, i.p., 1-4 h) decreased brain KYNA formation and furthermore, clearly potentiated the excitatory effect of clozapine.
Discussion: Our results show that the levels of brain KYNA are of importance for the response of clozapine on midbrain dopamine neurons and suggest that clozapine interacts with glutamatergic mechanisms. Furthermore, our data also suggest that levels of brain KYNA in schizophrenic patients are of importance for the therapeutic action of clozapine – this is of particular interest since patients with schizophrenia display increased CSF concentration of KYNA compared to healthy volunteers.

141. Preattentive Sensory Processing is Associated with Cognitive and Psychosocial Functioning in Healthy Adults
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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2005

Background: Understanding the basic neural processes that underlie complex higher-order cognitive operations and psychosocial functioning is a fundamental goal of cognitive neuroscience. Event-related potentials (ERPs) allow investigators to probe the earliest stages of information processing. Mismatch negativity (MMN) and P3a are auditory ERP components that reflect largely automatic task-irrelevant sensory discrimination. The aim of the present study was to determine if MMN and P3a are associated with higher-order cognitive operations and psychosocial functioning in clinically normal, healthy subjects.
Methods: Subjects included 20 normal adults who underwent comprehensive clinical assessments via structured interview to ensure that they were free of current and past Axis I disorders. All subjects were tested on a neurocognitive battery, underwent neurophysiological testing to assess MMN and P3a to task-irrelevant auditory stimulation, and ratings of psychosocial functioning.
Results: Subjects were within the normal range on cognitive tests and functional ratings. Across frontocentral electrode regions, significant correlations were observed between psychosocial functioning and MMN ($r=-0.62$, $p<0.01$) and P3a ($r=0.63$, $p<0.01$). P3a was also highly associated with immediate and delayed recall of verbal information with robust correlations widely distributed across frontocentral recording areas (e.g., $r=0.72$, $p<0.001$).
Discussion: Neurophysiological measures of relatively automatic and basic sensory processing are associated with psychosocial functioning and higher-order cognitive abilities and in normal subjects. Efficiency at elementary levels of information processing may underlie the successful encoding, retrieval, and discrimination of task-relevant information which in turn may well facilitate the iterative and responsive processing necessary for adaptive social functioning.

142. Sarcosine (N-Methylglycine) Treatment for Acute Schizophrenia
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Background: N-methyl-D-aspartate (NMDA)-enhancing agents have revealed benefits as adjuvant therapy for patients with schizophrenia. Among these compounds, sarcosine (a glycine transporter-I inhibitor) has been shown to be effective for both chronically stable and acutely-ill patients when added to antipsychotics. The present study aimed to test its efficacy and safety as the sole antipsychotic for acute schizophrenia by comparing two dosages: 2 grams vs. 1 gram/day, which is an ineffective lower dose.
Methods: Twenty acutely symptomatic drug-free patients with schizophrenia were enrolled in a 6-week double-blind trial of sarcosine. Clinical manifestations, quality of life, and side effects were evaluated every two weeks.
Results: Only the 2 gram, not 1 gram control, group improved at the end of the trial. The symptomatic improvements were in Scale for the Assessment of Negative Symptoms (SANS), Positive and Negative Syndrome Scale (PANSS). The 2 gram group was more likely to respond than the control group as defined by 20% reduction of the PANSS total score. Compared to the control, the 2 gram group was superior in improving scores in SANS, PANSS-total, -negative and -general, and Quality of Life. Among the 2 gram group, antipsychotic naïve patients responded more favorably than patients who had history of antipsychotic treatment. Both doses were well tolerated and no extrapyramidal symptoms or other significant side-effect was noted.
Discussion: Sarcosine therapy by itself at 2 grams/day shows comprehensive efficacy profiles. It has the advantage of not inducing any extrapyramidal symptom. Further placebo-controlled studies to compare sarcosine vs. antipsychotics and long-term studies are warranted.

143. The Atypical Paliperidone Induces Neurogenesis in Rat brain: A Controlled Study
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Background: Atypical antipsychotics have been shown in several studies to induce favorable neuroplastic changes that include neurogenesis (Wang et al, 2004). Atypical antipsychotics have also been demonstrated to be neuroprotective in schizophrenia subjects compared to the typical neuroleptic haloperidol (Lieberman et al, 2004). Paliperidone, the active metabolic of risperidone, is currently being considered by the FDA for the treatment of schizophrenia. We hypothesized that, like risperidone, paliperidone may be associated with

increased neurogenesis in rats. We conducted a controlled study to test this hypothesis.
Methods: Young male Sprague-Dawley rats were given 1 mg/kg/day of risperidone (N=6), 1 mg/kg/day of paliperidone (N=6) or diluent (N=6) in drinking water after obtaining approval by the Institutional Animal Use Committee. After 28 days of administration, animals were injected with bromodeoxyuridine (BrdU) 75 mg/kg each,1 and 2 hours prior to sacrifice, then perfused with 4% formaldehyde. Neural tissue was harvested, frozen sections were cut and every fifth section was stained with anti-BrdU. Positive cells were counted in stem cell areas of neural tissue (2-9 sections per animal).
Results: BrdU counts for each of the risperidone and paliperidone groups were significantly higher ($p<.05$) than controls. The Paliperidone and Risperidone groups did not differ statistically from each other.
Discussion: Paliperidone, like risperidone, was found to stimulate a significant increase in neurogenesis in rats compared to vehicle controls. We are currently conducting additional studies in several neural regions (including hippocampal, olfactory and subventricular) to confirm our findings, and also using different methodologies for administering the drug in addition to drinking water. The clinical implication of neurogenesis with atypical antipsychotics will be discussed .
References: 1. Wang et al. Neuropsychopharmacology 2004; 29:1230-8 2. Lieberman JA et al: Arch Gen Psychiatry 2005; 62:361-370

144. Is Clozapine Uniquely Effective in Treatment Resistant Schizophrenia?
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Background: Clozapine is the only antipsychotic drug (APD) with an indication for treatment-resistant (TR) schizophrenia, defined as persistent moderate-severe positive symptoms despite two or more trials of typical or atypical APDS at adequate dose and duration. We postulated that clozapine was found to be more effective than typical APDs in treating TR schizophrenia, which are ineffective at any dose in most patients with TR schizophrenia . (Kane et al. 1988) The benefits of the atypical APD could be due, in part, to its serotonergic/dopaminergic pharmacology and that, because of its lower EPS potential, related, in part, to its relatively more potent 5-HT2A than D2 antagonism, it could be used at significantly higher doses than is necessary for non-TR patients, which is in the 100-300 mg/day range (Fitton and Heel, Drugs 40: 722-740, 1990). We postulated that at least some other atypical APDs which are serotonin-dopamine antagonists, e.g. olanzapine and risperidone, would be as effective as clozapine in TR schizophrenia were they also to be used at higher doses, if tolerated, than are needed in non-TR schizophrenia and for longer periods of time.
Methods: We compared the doses of atypical APDs and plasma levels of clozapine that are effective in treating non-TR and TR patients. We carried out a double blind randomized, 6 month out-patient pilot trial in 39 TR patients comparing the effect of clozapine, at the higher dose range customary for TR schizophrenia, and high dose olanzapine (up to 45 mg/day) for up to six months. We also reviewed the literature on high doses of other APDs, including melperone, another atypical APD, which is a more potent 5-HT2A than D2 antagonist (Meltzer et al., Psychiatry Research 105: 201-9, 2001.) and risperidone.
Results: Clozapine dosage and plasma levels were found to be significantly less for non-TR clozapine responders than for TR clozapine responders. High doses of olanzapine (mean 33 mg/day) and clozapine (564 mg/day) produced non-significantly different improvement in positive and negative symptoms, as well as cognition, in a randomized double blind trial of 39 patients with TR schizophrenia (Meltzer et al., J Clin Psychiatry, in press). At six weeks, 18% of olanzapine-treated patients and 7% of clozapine-treated patients were treatment responders based on a priori criterion of a 20% or greater decrease in PANSS Total score. At six months, 50% of the olanzapine-treated group and 60% of the clozapine-treated group were responders by this criterion.Weight gain was significantly greater with olanzapine than clozapine. Similar differences in the doses needed for TR-and non-TR schizophrenia have been reported for melperone. Olanzapine, 10 mg/day, was recently reported to be as effective and better tolerated, in non-TR schizophrenia, than olanzapine 20 or 40 mg/day (Kinon et al. in preparation). Thus, the dosage of the atypical APDs for TR patients appears to be 2-4 times greater than that for non-TR patients.
Discussion: The evidence that TR patients with schizophrenia respond to atypical but not typical APDs at significantly higher doses and plama levels than non-TR patients and that longer treatment is needed for that response is modest at this point but of great importance clinically and theoretically, if it can be confirmed by further study. Similar results are to be expected with other pharmacologically related atypical APDs, e.g. risperidone. This delayed response may be confined to patients with structural deficits in cortex or hippocampus which are evident on CT (Friedman et al. Biological Psychiatry 29:865-877, 1991) or MRI, and which may be ameliorated, in part, by high doses of clozapine and related drugs which have neuroplastic benefits not found with drugs which are 5-HT2A antagonists with limited DA antagonist efficacy. The theoretical implications will be discussed.

145. Permissive Antagonism Induced by Novel Allosteric Antagonists of Metabotropic Glutamate Receptor 7
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Sponsor: Peter Jeffrey Conn

Background: A relatively recent concept that has developed within the field of G-protein coupled receptor (GPCR) pharmacology is ligand-directed trafficking of receptor signaling. It is now clear that distinct agonists can differentially activate coupling of GPCRs to various signaling pathways. These differences can range from full efficacy in one pathway to little or no activity in a second cascade known to be activated by the GPCR. In addition, these differences in efficacy are also dependent upon the context in which a signal is transduced; for example, there may be differences in compound activity between two distinct cell lines or between a cell line and an in vivo setting. For antagonists that competitively displace a receptor agonist from its binding site, all pathways activated by the agonist are blocked. In contrast, antagonists that act at an allosteric site could theoretically show "permissive antagonism" (reviewed in Kenakin, 2005, Nat Rev Drug Discovery, 4(11):919-927), blocking some signaling pathways induced by agonist binding while leaving other cascades intact. The interest in GPCRs as drug targets, coupled with the potential advantages of allosteric ligands as therapeutics, suggests that it is important to consider context-dependent effects of a given compound in relation to drug discovery.
Methods: We have explored the phenomenon of permissive antagonism using a series of allosteric antagonists of metabotropic glutamate receptor 7 (mGluR7). Based on studies with mGluR7 -/- mice, antagonists for mGluR7 are hypothesized to be potentially promising for the treatment of anxiety and depression. Nakamura et al. (2002) recently reported a series of isoxazolopyridone derivatives that had activity as antagonists of mGluRs (U.S. patent WO02102807).We screened a small library of compounds based on the structures of these antagonists for activity at mGluR7. For initial studies, we employed a fluorometric calcium assay using a recombinant cell line in which rat mGluR7 and the promiscuous G protein $G\alpha 15$ were co-expressed.
Results: Compounds in the series showed a broad range of inhibitory activity and potencies, with the most potent compounds

having IC50s in the 50 to 100 nM range. A subset of these mGluR7 antagonists were then examined for selectivity and displayed no activity when tested at 1 μM on rat mGluR4 and -8, indicating specificity for mGluR7 among the group III mGluRs. Using an alternate mGluR7 cell line (HEK/mGluR7) that lacks the promiscuous Gα15 protein and relies upon coupling through endogenous Gi/o-G proteins, we observed that these compounds do not block L-AP4-mediated inhibition of cAMP formation. Similarly, preliminary studies in the HEK/mGluR7 cell line indicate that these compounds do not affect L-AP4-stimulated GTPγS binding. These findings suggest that the results obtained from the Gα15-mGluR7 cell line might be specific to this receptor-G protein combination. We then assessed the ability of these compounds to inhibit mGluR7 coupling to G protein-gated inwardly rectifying K+ (GIRK) channels that were co-expressed in the HEK/mGluR7 line. Although approximately five fold less potent in this paradigm compared to the calcium assay, the compounds effectively blocked GIRK activity.
Discussion: These results suggest that these compounds show "permissive antagonism", blocking signaling through only some mGluR7-regulated signaling pathways. Future studies will use these compounds as tools to understand which signaling pathways are important for in vivo biological activity downstream of mGluR7. Supported by grants from NINDS, NIMH, and the Michael J. Fox Foundation. Vanderbilt is a center in the NIH supported MLSCN.

146. FGF-2 Acts as a Neurotrophic Agent, Activating ERK and Akt in Cultured Hippocampal Neurons

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Background: Fibroblast growth factor-2 (FGF-2) has been identified as having a key role in promoting hippocampal neurogenesis, and additionally appears to have neuroprotective actions in some models. It would therefore appear to be similar to IGF-1 and BDNF, neurotrophins which have been more extensively studied. In the current studies, cultured hippocampal neurons were utilized to directly compare the neurotrophic actions of FGF-2 to those of IGF-1 and BDNF.
Methods: Hippocampi were isolated from E18 rats, and neurons were cultured under serum-free conditions. The day prior to use, media was removed and replaced with media containing low (100ng/ml) concentrations of insulin. Cytosine arabinoside was included in culture media to prevent proliferation of non-neuronal cells. ERK and Akt activity were assessed using phospho-specific antibodies after gel electrophoresis. Cell survival was assessed 3 days after media change using MTT assays.
Results: All three agents promoted survival of neurons cultured under serum-free, low insulin conditions, with FGF-2 being significantly more effective than IGF-1 and BDNF. FGF-2 also stimulated the largest magnitude of Akt and ERK activation. Combined treatment with maximal concentrations of FGF-2 and IGF-1 resulted in approximately additive increases in both Akt and ERK activation. In contrast, co-treatment with maximal concentrations of BDNF and FGF-2 stimulated approximately additive increases in Akt activity, but less than additive increases in ERK activity. Co-treatment with maximal concentrations of FGF-2 and either IGF or BDNF stimulated less than additive increases in neuronal survival. In summary, FGF-2 was found to be a more effective neurotrophic agent than the better characterized IGF-1 and BDNF. Additionally, growth factors, when used in combination, elicited larger increases in ERK and Akt activity than that stimulated by single neurotrophins.
Discussion: Antidepressants have been found to increase the level of IGF-1, FGF-2, and BDNF. These findings may therefore be relevant to understanding the neuroprotective actions of antidepressants in the hippocampus.

147. The "Selective" 5-HT1A Antagonist WAY-100635 and Its Metabolite, WAY-100634, Are Potent Dopamine D4 Receptor Agonists

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Background: WAY-100635, the prototypical 5-HT1A receptor antagonist, has been widely used as a pharmacological probe to investigate the distribution and function of 5-HT1A receptors. Results from in vitro studies in our laboratory suggested that WAY-100635 was potent in producing effects unrelated to its 5-HT1A receptor affinity. In the present work we evaluated the in vitro pharmacology of WAY-100635 at two dopamine D2-like receptor subtypes. We also carried out a preliminary characterization of the interoceptive cue produced by WAY-100635 in drug discrimination studies in rats.
Methods: The binding affinities and functional properties of WAY-100635 were studied in HEK 293 cells stably expressing human dopamine D2L or D4.4 receptors. Initial screening was performed by the NIMH Psychoactive Drug Screening Program. For drug discrimination tests, male Sprague-Dawley rats were trained to discriminate WAY-100635 (10 micromoles/kg) from saline in a food-reinforced FR50 two-lever drug discrimination paradigm.
Results: The NIMH PDSP screening found that WAY-100635 displayed 940 nM, 370 nM, and 16 nM binding affinities at D2L, D3, and D4.2 receptors, respectively. Subsequent saturation analyses using [3H]WAY-100635 gave a Kd of 2.4 nM at D4.2 receptors, only 10-fold higher than its affinity at the 5-HT1A receptor. Both WAY-100635 and its major metabolite, WAY-100634, were potent agonists in HEK-D4.4 cells (EC50 = 9.7 and 0.65 nM, respectively). WAY-100635 was a full agonist, and WAY-100634 behaved as a nearly full agonist. In HEK-D2L cells, WAY-100635 was a weak antagonist of the effects of 300 nM quinpirole. Radioligand competition studies with [3H]spiperone confirmed that WAY-100635 possesses high affinity for D4.4 receptors, but binds only weakly to D2L receptors (3.3 and 420 nM, respectively). In the drug discrimination study, rats readily learned to discriminate WAY-100635, in contrast to the near impossibility of training with most types of known GPCR antagonists. Tests of the nature of the cue with selective dopamine D4 agonists and antagonists revealed that the WAY-100635 cue was mediated by agonist activity at the dopamine D4 receptor.
Discussion: These studies demonstrate that WAY-100635 is not a "selective" 5-HT1A receptor antagonist. Conclusions drawn from studies that employed WAY-100635 as a selective 5-HT1A antagonist may need to be re-evaluated. This work was supported by NIH grant DA02189 (DEN & VJW), MH60397 (VJW), and the NIMH PDSP, and KO2MH01366 (BLR).

148. Ethanol Potentiation of D1 Dopamine Receptor Signaling Can be Mediated by Protein Kinase C in an Isozyme-Specific Fashion

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Background: Ethanol consumption is well known to potentiate dopaminergic signaling and this is partially mediated by the D1 receptor. The mechanism responsible for ethanol modulation of D1 receptor signaling is unclear and is the focus of our current study. We have found that ethanol pretreatment of D1 receptor-transfected HEK293 cells potentiates dopamine-stimulated cAMP accumulation and decreases D1 receptor phosphorylation without altering receptor expression. We hypothesize that ethanol may decrease D1 receptor phosphorylation and enhance signaling by either activating a protein phosphatase or inhibiting a protein kinase.
Methods: To examine the potential involvement of phosphatases or kinases on D1 receptor phosphorylation and signaling, HEK293 cells

expressing the D1 receptor were pretreated with several phosphatase or protein kinase C (PKC) inhibitors prior to dopamine-stimulation. D1 receptor-mediated signaling was evaluated using cAMP accumulation assays and D1 receptor phosphorylation was assessed via in situ phosphorylation assays.
Results: Pretreatment with phosphatase inhibitors did not abolish the ethanol potentiation of dopamine-stimulated cAMP levels or the decrease in D1 receptor phosphorylation. Furthermore, co-expression of the D1 receptor with a constitutively active subunit of calcineurin (protein phosphatase 2B) did not potentiate dopamine-stimulated cAMP levels or reduce basal D1 receptor phosphorylation levels when compared to control cells expressing the D1 receptor alone. However, cellular pretreatment with PKC inhibitors mimicked the effects of ethanol on both dopamine-stimulated cAMP levels and D1 receptor phosphorylation, suggesting that ethanol functions to inhibit basal PKC phosphorylation of the receptor. This idea is supported by the observation that treatment of the cells with both ethanol and PKC inhibitors promote non-additive effects on D1 receptor phosphorylation and activity. In cells cotransfected with the D1 receptor and the PKC isozymes γ or δ, the ethanol-dependent decrease of D1 receptor phosphorylation appears to be augmented suggesting that the effects of ethanol may be mediated by these PKC isozymes. The ability of ethanol to modulate PKC activity in the cells was directly assessed using vitro kinase assays following selective immunoprecipitation of specific PKC isozymes. We found that ethanol pretreatment of the cells indeed attenuated the membrane kinase activities of PKCγ and PKCδ whereas those of PKCβ and PKCε were unaffected.
Discussion: Taken together, these results suggest that PKCγ and PKCδ constitutively phosphorylate the D1 dopamine receptor under basal conditions and that this dampens receptor-G protein coupling. Exposure to ethanol specifically inhibits the activity of these PKC isozymes resulting in decreased basal receptor phosphorylation and enhanced D1 receptor-mediated signaling.

149. Blockade of Glutamatergic Receptors Decreases Consumption of a Standard Food Diet and a Preferred Food in Non-Human Primates

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Sponsor: Richard Foltin

Background: Glutamatergic pathways are involved in regulating consumatory behaviors and may therefore be a target for eating disorders medication development. We have studied the effect of acute administration of a NMDA glutamatergic receptor antagonists, memantine (MEM), in a model of binge eating in laboratory animals. For comparison, the effect of the prototypic anorectic agent d-fenfluramine (DFEN) was also studied.
Methods: Four male and four female young adult baboons had unrestricted access to water and were never food deprived. On test days, animals could respond under two-phase chain schedule of reinforcement to receive one of two types of food: regular food pellets and a highly-palatable (HP) food (hard-coated jelly candy). Three days a week (M, W, F), the day started with access to a single candy meal that continued until the baboon stopped responding for 10 min, after which baboons had access to pellet meals until the following morning. Only pellet meals were available on the remaining 4 days each week. On test days (M,Th) baboons received an i.m. injection of the study drug prior to the session. MEM and DFEN were tested at doses 0, 0.25, 0.50, 1.0, and 2.0 mg/kg.
Results: On average, animals ate about 165 food pellets on pellet-only days. When candy was available, animals ate about 145 candies and 100 pellets. The latency to the first pellet meal on pellet-only days was about 2 hr, while the latency to the first candy meal was about 1 hr. None of the test drugs altered the latency to the candy meal but all of them produced a dose-dependent decreases in the number of candies consumed during a candy-binge meal. At the highest doses, MEM and DFEN reduced the size of the candy-meal to approximately 45% and 30% respectively of candies intake under vehicle condition. In comparison, both drugs produced a dose-dependent increase in latency to the first food pellet meal, by up to 5 hours, and a dose-dependent decrease in the size of pellet meal. In addition, both drugs decreased the number of daily pellet meals.
Discussion: Present results suggest that NMDA receptor-mediated neurotransmission may be involved in regulating binge-type consumption of HP foods. The latency to begin the HP food meal was not altered, but its reinforcing effects to maintain continuous consumption was decreased through after treatment with NMDA receptor antagonists. This may represent an enhancement of satiety and an earlier termination of a HP binge without affecting the appetite. In the present study, the effect of MEM was comparable to a clinically effective anorectic and anti-binge agent DFEN. These findings suggest that glutamatergic receptor antagonists might be promising as pharmacotherapies for eating disorders. It is worth noting however that MEM does not have anorectic effects when used in elderly patients with dementia, therefore this effect may be different depending on the population.

150. Trauma, Coping Strategies and Resilience in African Americans

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Background: While psychiatric disorders increase after trauma, they are not inevitable consequences of trauma exposure. Varied definitions of resilience include the capacity to thrive in the face of adversity, the absence of psychiatric disorders after trauma exposure and the capacity to recover from psychiatric disorders over time. The study of populations at high risk for trauma provides a unique opportunity to examine coping strategies associated with various levels of resilience to trauma. Few studies have addressed coping and resilience after trauma in African Americans. We hypothesized that positive coping strategies would be associated with resilience and recovery, and negative coping strategies with current psychiatric illness in this population.
Methods: Study participants were recruited from primary care offices at Howard University Hospital. Patients were approached while waiting to see their doctor and asked to complete a self-report questionnaire inquiring about their history of trauma exposure (Life Events Checklist from the CAPS), after obtaining informed consent. All patients who identified at least one significant traumatic event were invited to participate in an in-depth diagnostic assessment that included the SCID-IV and the CAPS, conducted by trained clinical interviewers. Patients also completed the COPE, a self-report instrument that assesses 12 different coping strategies (Carver et al 1999). Of a total of 737 patients surveyed, 584 met criteria for trauma exposure, 352 participated in diagnostic interviews, and 327 had complete data. After excluding patients with bipolar or psychotic disorders, the final sample included 260 patients. Patients were assigned to one of three groups: a resilient group with no lifetime psychiatric disorders (n = 53), a recovered group with at least one past but no current disorder (n = 79), and a currently ill group with at least one current disorder (n = 128). Logistic regression analyses were conducted with the three pairs of groups as dependent variables (resilient vs. ill, recovered vs. ill, and resilient vs. recovered) and demographic variables (gender, age, education), trauma type (assaultive vs. non-assaultive), and coping strategies as predictors.
Results: The total sample of 260 patients was 67.3% female and predominantly African American (96.9%). Mean age (SD) was 42.1 (13.6) years. The majority of patients had graduated from high school (79.2%) and 44.6% had attended at least some college. The

most frequently reported trauma types were transportation accidents (51.2%), physical assault (39.2%), assault with a weapon (36.5%) and sexual assault (34.6%). The mean (SD) number of traumas per patient was 3.5 (2.2). Results indicated that female gender ($p = .003$), history of assaultive trauma ($p = .001$) and higher behavioral disengagement ($p = .015$) significantly predicted currently ill vs. resilient group status. Higher behavioral disengagement also significantly predicted currently ill vs. recovered status ($p = .008$), while higher active coping significantly predicted recovered vs. currently ill status ($p = .046$). Higher acceptance significantly predicted recovered vs. resilient status ($p = .048$).
Discussion: Consistent with findings in other populations, female gender and a history of assaultive trauma were associated with a higher probability of being currently ill. While we cannot infer causality, findings in African Americans suggest that active coping is important for recovery, while behavioral disengagement (giving up) might prolong psychiatric illness. Further, acceptance might be more important for recovery than for maintaining resilience from the start. Prospective studies could help further evaluate whether certain pre-existing coping styles are predictive of resilience and recovery after trauma.

151. cTMS: A Novel TMS Device Inducing Near Rectangular Pulses with Controllable Pulse Width

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Background: Transcranial Magnetic Stimulation (TMS) is a useful tool for probing brain function, and has shown promise as a potential treatment for depression and other disorders. The physiological response to TMS depends on the shape and width of the induced current pulses, however, existing TMS devices allow limited, if any, control over these parameters. Conventional TMS devices produce sinusoidal pulses with fixed pulse width. We have developed a novel monophasic TMS device (cTMS1) that induces near rectangular pulses with continuous control over pulse width. Pulse width control could be used for selective targeting of neuronal populations with different chronaxie. For example, very brief pulses may reduce scalp sensation, thus improving tolerability and blinding for clinical trials. The cTMS1 device can also be used to study neuronal membrane time constants, which characterize membrane thickness, myelination, and state of the ion channels of different neuronal population in healthy and diseased states, and under pharmacological interventions. We designed and bench tested the cTMS1 device, and used it to measure the motor cortex neuronal membrane time constant of the rhesus monkey.
Methods: Electrical Tests: cTMS1 was tested with capacitor voltages up to 1650 V, and peak coil currents up to 5 kA, matching the parameter range of conventional Magstim Rapid TMS stimulators. The cTMS1 output was characterized by measuring the electric field induced in a search coil. In Vivo Tests: Five male Macaca mulatta (age 9.4±2.9 yr, weight 10.7±2.7 kg (mean±s.d.)) were sedated with ketamine 5.0 mg/kg and xylazine 0.3 mg/kg im. Physiological monitoring included ECG, pulse oximetry, end-tidal pCO2, and blood pressure. A 4 cm diameter round coil was placed at vertex. Resting motor threshold (MT), defined as the minimum stimulation intensity yielding at least 5 of 10 motor evoked potentials with peak-to-peak amplitude > 50 μV, was measured for the left first dorsal interosseous muscle at five pulse widths (20, 40, 60, 80, and 100 μs) presented in random order.
Results: Electrical Tests: To date cTMS1 has produced over 2,000 pulses during bench and in vivo tests. Pulse width can be continuously adjusted between 10 and 160 μs. The output has amplitude error of less than 3%, and pulse width error of less than 0.4%. In Vivo Tests: All procedures were well tolerated with no adverse events and no change in vital signs. As expected, MT decreased as the pulse width increased. For each subject the neuronal time constant was estimated from the strength-duration data with a non-linear least-squares curve fitting method. For the curve fitting algorithm, recorded electric field pulses were used in conjunction with a first-order low-pass filter model of the neuronal membrane to derive the relationship between membrane time constant and peak membrane voltage for the measured pulse widths. The neuronal membrane time constant was estimated to be 116±25 μs (mean±s.d.), which is close to the published mean value of 150 μs for human motor cortex.
Discussion: These findings demonstrate the feasibility of constructing a cTMS device capable of controllable pulse width with near rectangular pulse shape, and illustrate an application of pulse width control to noninvasively measure membrane time constants. Future work will examine the utility of cTMS for selective targeting of neuronal populations and improving the tolerability of TMS. This work was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

152. Regional Expression of Cytochrome C Oxidase in the Hippocampus Following Long Term Potentiation at the Mossy Fiber–CA3 Synapse

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Background: Our laboratory previously used microarray technology to identify changes in gene expression in the hippocampus of adult rats following long-term potentiation (LTP) at the mossy fiber (MF) – CA3 synapse. One gene that was significantly upregulated following LTP was cytochrome c oxidase (COX), a mitochondrial protein involved in the synthesis of adenosine triphosphate (ATP). Since ATP is the primary energy source of all cells and is synthesized in part by COX activity, COX expression is considered to represent metabolic demand. In the present study we used in situ hybridization to localize the expression of COX in the hippocampus of animals that received electrophysiological stimulation to the MF-CA3 pathway.
Methods: Adult male Sprague-Dawley rats were divided into an experimental group (LTP, n=3) and two control groups (high frequency stimulation (HFS) (n=2), and low frequency stimulation (LFS) (n-2)). LTP at the MF-CA3 synapse is opioid dependent. Hence, immediately after a 20-minute baseline recording (0.05 Hz pulse delivered every 20 seconds) and one-hour prior to tetanus (two one-second 100Hz pulses) animals in the HFS group were given the μ-opioid antagonist, naloxone, to ensure the electrodes were placed in the MF pathway. Our data indicate that naloxone effectively inhibited LTP thus verifying our electrode placement. To control for any effects due to drug administration, animals in the LTP group were given a systemic injection of the NMDA receptor antagonist CPP 20-minutes after baseline and one-hour prior to tetanus, which did not affect MF-CA3 LTP induction. Finally, animals in the LFS group were given saline vehicle following a 20 minute baseline and did not receive tetanic stimulation. In the LTP and HFS groups the EPSPs were recorded for one hour following tetanus, and for animals in the LFS group, EPSPs were recorded for one hour following the 20-minute baseline. Following electrophysiology the brains were removed, immersed in methyl butane and stored at -80oC. Nine μm slices throughout the dorsal hippocampus were mounted onto slides and processed for in situ hybridization using a radiolabeled COX probe.
Results: Analysis of Variance (ANOVA) revealed that animals in which LTP was induced exhibited a greater degree of COX expression when compared to HFS and LFS animals, and that the expression was especially robust in the CA3 apical dendrites.
Discussion: These results suggest that the changes in hippocampal COX expression following MF-CA3 LTP, as identified previously by our microarray analysis, are due to regional changes in COX activity in the CA3 region of the hippocampus. Furthermore, our data indi-

cate that LTP at the MF-CA3 synapse is a metabolically demanding process, and that in the CA3 region COX expression is pronounced at the site where MFs synapse onto pyramidal cells.

153. Kinetic Analysis of Methylphenidate-Induced Changes in VMAT-2 Function Using Rotating Disk Electrode Voltammetry

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Background: It has been long recognized that a psychostimulant used commonly to treat attention deficit disorder, methylphenidate (MPD), inhibits dopamine (DA) transporter function. In addition to this effect, recent studies utilizing [^{3}H]monoamine uptake indicate that MPD increases vesicular [^{3}H]DA uptake, as assessed in a purified non-membrane-associated (presumably cytoplasmic) subcellular vesicular fraction prepared from treated rats. This effect reflects a redistribution of vesicular monoamine transporter-2 (VMAT-2), and presumably associated vesicles, from neuronal membranes into the cytoplasm. Recent studies from this laboratory have been the first to employ rotating disk electrode (RDE) voltammetry to characterize the real-time non-radiolabeled DA uptake profiles in these non-membrane-associated vesicles, and have demonstrated that these follow mixed-order kinetics with apparent zero order kinetics for approximately the first 25 s and apparent first order kinetics thereafter. Moreover, this vesicular DA uptake was ATP- and temperature-dependent. It was also blocked by the VMAT-2 inhibitor, tetrabenazine.
Methods: Having characterized the basic kinetics of transport, the purpose of the present study was to apply RDE voltammetry to characterize the impact of drug treatment with MPD on these non-membrane-associated vesicles.
Results: Results revealed that consistent with the [^{3}H]monoamine uptake studies, MPD treatment rapidly increased the density of kinetically active VMAT-2 (saline 0.36 ± 0.04 vs. MPD 0.55 ± 0.02 fmol/μg protein) and the Vmax (saline 3.7 ± 0.1 vs. MPD 5.7 ± 0.3 fmol/(s * μg protein)) of non-membrane-associated vesicular DA uptake without affecting K_m (~ 300 nM). Neither the transporter turnover number, k_{cat} (~ 10 s-1), nor the rate constant for DA binding to the VMAT-2, k_{ass} (~3.2 x 107 M-1 s-1), were affected by MPD treatment. This suggests that the MPD-induced increase in cytoplasmic DA uptake is caused solely by VMAT-2 trafficking to the cytoplasm and not by a change in the kinetics of transporter function.
Discussion: These data represent the first use of RDE voltammetry to assess VMAT-2 function ex vivo following in vivo drug administration. The implications of enhanced MPD-induced DA sequestration in cytoplasmic vesicles with regard to neurodegenerative diseases such as Parkinson's disease will be discussed. (This research was supported by DA11389, DA04222, DA00869, DA00378, DA019447, and a Focused Funding Grant from Johnson and Johnson.)

154. Effect of Long-Term Methylphenidate Treatment on Gene Expression in Various Brain Regions

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Methylphenidate (Ritalin®) is a psychostimulant used clinically for the treatment of narcolepsy and attention-deficit hyperactivity disorder. This drug is being prescribed to an increasing number of children in the United States, with treatment durations that often extend from childhood into adolescence and adulthood. Despite the extensive use of this stimulant medication, relatively little is known regarding the long-term consequences of methylphenidate exposure in the CNS. The therapeutic action of methylphenidate is believed to be due to its action as an indirect agonist of the dopamine transporter and to a lesser extent of the norepinephrine transporter. Therefore, we hypothesized that long-term methylphenidate treatment may alter gene expression in neuronal populations sending or receiving monaminergic input.
Methods: Methylphenidate (10 mg/kg/day) or saline was administered to juvenile (25 day old) male Swiss-Webster mice using a school week (5 day week) dosing regimen. Following 12 weeks of methylphenidate treatment, animals were held in a methylphenidate-free environment for 2 days prior to sacrifice. The brain regions examined included frontal cortex, hippocampus, substantia nigra, striatum, and the ventral tegmental area. RNA from each of these samples was isolated, and microarray analyses were performed using the M430v2 Affymetrix short oligomer microarrays. We classified genes as significantly different if they were up- or downregulated by at least 1.6-fold and had a p value less than 0.01.
Results: RNA isolated from the dopaminergic neuron-rich areas of the ventral tegmental area and the substantia nigra exhibited the greatest number of significant changes (69 genes upregulated, 231 genes downregulated and 122 genes upregulated, 47 downregulated, respectively), while smaller numbers of changes were seen in the other areas examined (frontal cortex: 18 upregulated, 32 downregulated; hippocampus: 8 genes upregulated, 8 genes downregulated; striatum: 10 genes upregulated, 19 genes downregulated). Surprisingly, the sets of up- and downregulated genes in each area examined were unique, and there were only a few cases where there was a common change.
Discussion: While several previously published studies have examined gene expression changes following acute dosing regimens of methylphenidate, we have examined changes that occur following a more clinically relevant schedule of methylphenidate administration. These studies may provide insight into the functional consequences of long-term clinical use of methylphenidate in the CNS.

155. Developing Drosophila Melanogaster as a Model Organism to Study Molecular and Genetic Factors Underlying Neuropsychiatric Disorders

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Sponsor: David E. Nichols

Background: Neuropsychiatric disorders like schizophrenia are debilitating diseases characterized by dramatic alterations in normal behaviors. Investigations into the molecular mechanisms underlying these disorders have traditionally involved studies in animal models. Unfortunately, the use of traditional mammalian models has a major drawback: there are serious limitations on genetic studies designed to elucidate molecular pathways and molecules relevant to behaviors. The elucidation of molecular signaling pathways leading from drug interactions at specific receptor targets to behavior are cumbersome, at best. These experiments often take years and require significant resources to identify a single component. One answer to this dilemma is to develop a genetically tractable model system to investigate these molecular mechanisms. The fruit fly, Drosophila melanogaster, is believed to express functional orthologs of the mammalian 5-HT2, 5-HT1A, 5-HT7, dopamine D1 and D2, GABA, NMDA, and metabotropic glutamate receptors, all strongly implicated in a variety of human neuropsychiatric disorders. Remarkably, Drosophila respond to agents that influence neurotransmitter function with complex behaviors in a manner very similar to what occurs in both rodents and primates. The advantages and power of Drosophila to study these processes include not only increased rate of discovery and reduced costs but, significantly, being able to follow a systems-based approach.
Methods: The effects of serotonergic and dopaminergic agents on behaviors relevant to neuropsychiatric disorders have been investigated in the fly, and assays developed to quantify these effects. These include studies to examine startle response, aggression, circadian rhythms, repetitive motion, and overt activity levels.

Results: We have found that these behaviors in the fly are differentially influenced by pharmacological agents. For example, the 5-HT2 receptor antagonist ketanserin increased startle response, while the 5-HT2 receptor agonist DOI attenuated it. Startle response was also attenuated by cocaine. The 5-HT1A/7 receptor agonist 8-OH DPAT dramatically increased aggression, while other drugs reduced only specific subsets of aggressive behaviors. For example, the 5-HT1A receptor antagonist WAY100635 primarily only affected boxing/tussling behaviors. Repetitive grooming behaviors were dramatically increased by the serotonin receptor antagonist mianserin. Mianserin also nearly abolished circadian behaviors without significantly affecting overt activity.
Discussion: A variety of complex behaviors in humans relevant to neuropsychiatric disorders including startle response, aggression, circadian rhythms, and stereotypy, are present in Drosophila. Here, we demonstrate that these behaviors can be influenced by drugs that alter serotonergic and dopaminergic function. Because the effects of these agents can be very specific towards one aspect of a behavior, we can begin to dissect individual receptor contributions to these behaviors. This work lays the foundation for future studies to elucidate the molecular and genetic pathways linking receptor activation to these behaviors. Significantly, the molecular pathways linking receptor interactions to these behaviors are very likely, as with most studied pathways, to be highly conserved between fly and human. Because of these parallels, the powerful genetic approaches developed in flies will provide a facile avenue to fast and sophisticated studies that may provide important insights that can be translated to an understanding of mammalian neuropharmacology and neuropsychiatric disorders, and ultimately to novel therapeutics to treat them.

156. Association of a Functional Variant in the Mu-Opioid Receptor Gene (*Oprm1* C77g) with Attachment Behavior in Infant Rhesus Macaques

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Sponsor: Past Travel Award, Young Investigator Memorial, 2004

Background: The endogenous opioid system has been demonstrated to be important in establishing and maintaining social attachment and is proposed to be important in ensuring infant survival. Mu-Opioid receptor gene polymorphisms that produce 3.5-fold increases in receptor affinity for β-Endorphin have arisen independently in humans and rhesus macaques and may confer some selective advantage in both species by influencing social attachment. We wanted to determine whether the gain-of-function *OPRM1* C77G polymorphism was associated with increased attachment behavior in infant rhesus macaques.
Methods: At six months of age, infants were subjected to four weeks of repeated maternal separation (96 H) and mother-infant reunion (72 H). Focal behavioral data were collected during baseline, acute and chronic separation, and reunion phases across the 4 weeks of the study. Animals were genotyped for the *OPRM1* C77G polymorphism (n = 99), and the effects of the *OPRM1* G allele on attachment-related behaviors were assessed as a function of the recurrence of maternal separation, using repeated measures ANOVA.
Results: There was an association of the G allele with higher rates of distress vocalizations with repeated maternal separation ($p = 0.03$). When infants were observed during mother-infant reunion, 77G allele carriers spent more time in mutual ventral contact with their mothers ($p = 0.02$) and were less likely to leave their mothers to explore the environment ($p = 0.02$) or to interact with other individuals in the social group ($p = 0.02$). This, again, was especially true after repeated exposure to maternal separation and was independent of maternal genotype.
Discussion: These findings are consistent with those demonstrating *Oprm1* gene disruption to diminish attachment behavior in mice and suggest that variation at the *OPRM1* locus may be associated with differences in attachment behavior in human subjects, especially as a function of separation from the caregiver. This research was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

157. Effect of Atypical Antipsychotics on Prolactin Levels and Reproductive Functioning in Children and Adolescents

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Sponsor: John M. Kane

Background: Atypical antipsychotics (AAs) are heterogeneous in their effects on prolactin and sexual functioning. However, little is known about these effects during development. While risperidone has consistently shown adverse prolactin changes, the relationship between prolactin elevations and clinical symptoms remain unclear. Furthermore, the effects of a partial dopamine agonist on prolactin and related adverse events in youth are unknown. Therefore, we aimed to assess the effects of 5 non-Clozapine AAs on prolactin and prolactin related side effects in youth.
Methods: As part of an ongoing, observational study in youths (4-19 years) treated with AAs for psychotic, mood and aggressive spectrum disorders, fasting AM prolactin and sexual side effects were measured within 7 days of AA initiation and monthly for the first 3 months. Patients switched to a different AA could be followed on the new AA.
Results: Of 478 enrolled youngsters, 378 (79.1%) patients (mean age: 13.4±3.6 years, 63.2% postpubertal, 60.3% male, 47.5% Caucasian) with mood disorders (44.6%), disruptive behavior disorders (29.2) and schizophrenia spectrum disorders (26.3%) had at least one post-baseline assessment. Data from 511 antipsychotic trials (mean duration: 10.7+/-3.3 weeks) included aripiprazole (n=116), olanzapine (n=81), quetiapine (n=104), risperidone (n=176) and ziprasidone (n=34). Hyperprolactinemia (>25.7 ng/mL) was present in 84.1% of youngsters on risperidone, 52.9% on ziprasidone, 48.1% on olanzapine, 14.4% on quetiapine, and 9.5% on aripiprazole ($p<0.0001$). On the other hand, hypoprolactinemia (<3.4 ng/mL) was present in 45.7% of youths on aripiprazole, 1.0% on quetiapine and none on olanzapine, quetiapine or ziprasidone ($p<0.0001$). Incidence rates of sexual side effects were 19.9% with risperidone, 15.8% with olanzapine, 10.5% with ziprasidone, 9.0% with quetiapine, and 7.3% with aripiprazole ($p=0.013$). In postpubertal females, oligomenorrhea or amenorrhea occurred in 30.0% with risperidone, 18.7% with ziprasidone and 17.4% with olanzapine, while no such cases existed in the quetiapine and aripiprazole groups. In multivariate regression analyses, hyperprolactinemia was significantly associated with risperidone ($p<0.0001$), olanzapine ($p<0.0001$), ziprasidone ($p<0.0001$), female sex ($p<0.0001$) and older age ($p=0.0002$) [r squared: 0.36, $p<0.0001$]. In multivariate regression analyses, hypoprolactinemia was significantly associated with prepubertal status ($p<0.0001$) and aripiprazole treatment ($p<0.0001$) [r squared: 0.62, $p<0.0001$].
Discussion: In children and adolescents, prolactin abnormalities are common with AA treatment. The frequency of sexual side effects in each AA group follows the ranking regarding the prolactin elevating properties. As in adults, risperidone has the greatest risk for hyperprolactinemia in the first 3 months of treatment. However, ziprasidone and olanzapine are also associated with abnormally elevated prolactin levels in up to half of patients. Quetiapine and, especially, aripiprazole are associated with hyperprolactinemia the least. However, particularly in prepubertal children, aripiprazole is associated with hypoprolactinemia, confirming its dopamine agonist activity. Further research is needed to track these changes over longer periods of time. In addition, research is needed to assess the relevance of ab-

normally elevated or abnormally decreased prolactin levels on sexual functioning and maturation, as well as on other physiological functions that are under the influence of prolactin, including bone mineralization, immunity and metabolic health.

158. The Mechanisms of Habit: Analysis of Striatum-Dependent Procedural Learning in Wild-Type and CREB Transgenic Mice

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Sponsor: Travel Awardee, Young Investigator Memorial, 2006

Background: Repeated actions can take on a habitual character. A habit is acquired over multiple repetitions, is executed in a relatively stereotyped fashion, and requires little or no conscious effort or attentional resources to execute but some effort to interrupt. Acquisition and execution of such learned, stereotyped patterns of behavior involves the basal ganglia. A number of neuropsychiatric disorders in which basal ganglia function is perturbed may be understood, at least in part, in terms of habits gone awry. This is most clearly true in the case of Tourette's syndrome, obsessive-compulsive disorder, and drug addiction. An increased understanding of basal ganglia-dependent procedural learning is likely to shed light on these and related disorders. While the neuroanatomical substrates of habit learning are increasingly well delineated, the underlying cellular and molecular mechanisms are incompletely understood. The analysis of striatum-dependent learning processes in genetically modified mice is a powerful tool for advancing this understanding. We have recently published a study of striatum-dependent procedural learning in transgenic mice expressing a CREB dominant-negative transgene in the dorsal striatum. We now report the extension of this work into more and better-validated striatum-dependent learning tasks in mice.
Methods: Two striatum-dependent learning tasks are described. In the first, a cue-discrimination watermaze task, animals learn to locate a platform onto which they can escape from a pool of water. This task has the advantage of allowing hippocampus-dependent and striatum-dependent learning strategies, with identical performance requirements, to be probed in parallel. In the second task, instrumental habit in the operant chamber, animals are trained to lever-press for food using a random-interval training paradigm. In this task the habitual nature of responding can be rigorously probed by assaying its resilience to reinforcer devaluation. Both of these tasks have been developed and their dependence on the dorsal striatum clearly demonstrated in rats, but they have not previously been explored in mice. First, wild-type animals are tested, to confirm their ability to learn and to explore some of the learning parameters. Second, animals with excitotoxic dorsal striatal lesions are tested, to rigorously evaluate the dependence of both categories of learning on the function of this structure. Finally, transgenic animals expressing a CREB dominant-negative transgene in the dorsal striatum are assayed, to probe the role of CREB-mediated processes in the relevant modes of information storage.
Results: Mice are able to learn both tasks. Their performance is similar to that of rats in comparable tasks and is consistent with a critical role for the dorsal striatum in these forms of procedural learning in mice. Ongoing studies are probing the involvement of the dorsal striatum in these tasks in mice and their dependence on striatal CREB in transgenic animals.
Discussion: Striatum-dependent procedural learning is likely to be important both to normal function and to the understanding of certain forms of basal ganglia pathology. The two types of learning tasks described here derive from different literatures and have rarely been examined in parallel; the mechanistic and neuroanatomical similarities and differences between them are therefore not well understood. Examination of such learning tasks in genetically modified mice is likely to shed light both on the mechanisms of striatum-dependent learning in general and the similarities and differences between different subtypes of such learning. Our experiments represent an important first step in this direction, probing the role of CREB regulated processes and laying the groundwork for future mechanistic studies.

159. Vitamin D Deficiency Is Associated with Low Mood, Worse Cognitive Performance and Smaller Hippocampal Volumes in Older Adults

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Sponsor: Yvette I. Sheline

Background: Vitamin D deficiency is common in older adults and has been implicated in psychiatric and neurologic disorders. This study examined the relationship between vitamin D status, cognitive performance, mood, and hippocampal size in older adults.
Methods: A cross-sectional group of 80 participants, 40 with mild Alzheimer's disease (AD) and 40 nondemented controls, were selected from a longitudinal study of memory and aging. Cognitive function was assessed using the Short Blessed Test (SBT), Mini-Mental State Exam (MMSE), Clinical Dementia Rating (CDR; a higher Sum of Boxes score indicates greater dementia severity) and a factor score from a neuropsychometric battery; mood was assessed using clinician's diagnosis and the depression symptoms inventory. Serum 25-hydroxyvitamin D levels were measured for all participants. A subset of the group, 22 nondemented persons, underwent volumetric MRI. Hippocampal volumes were measured using stereological estimation methods.
Results: The mean vitamin D level in the total sample was 18.58ng/ml (SD 7.59); 58% of the participants had abnormally low vitamin D levels, defined as less than 20ng/ml. After adjusting for age, race, gender and season of vitamin D determination, vitamin D deficiency was associated with presence of an active mood disorder ($p = .022$, OR: 11.69; 95% CI: 2.04-66.86). Using the same covariates in a linear regression model, vitamin D deficiency was associated with worse performance on the SBT ($p=.044$), and higher CDR Sum of Box scores ($p = .047$) in the vitamin D deficient group. There was no difference in performance on the MMSE or factor scores between the vitamin D groups. Left hippocampal volumes (mm3) were smaller in the vitamin D deficient group, 2845 mm3 (SD 508), compared to the vitamin D sufficient group, 3592 mm3 (SD 738), ($F=5.05$, $p=0.044$).
Discussion: In a cross-section of older adults, vitamin D deficiency was associated with low mood and impairment on two of four measures of cognitive performance. Nondemented persons with vitamin D deficiency also had smaller hippocampal volumes. This supports a possible role of vitamin D deficiency in low mood and cognitive impairment.

160. Challenges in the Pharmacologic Characterization of the Trace Amine-Associated Receptor 1 (TAAR1)

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Sponsor: J. David Leander

Background: Since the 1970s it has been hypothesized that so-called trace amines found in the brain could be involved in both normal and pathological processes. However, the study of this was severely hampered by the lack of knowledge of the potential targets of the trace amines and the lack of pharmacologic tools. The initial cloning of a family of putative trace amine receptors in 2001 (Borowsky et al., Proc Natl Acad Sci USA 98:8966, 2001; Bunzow et al., Mol Pharmacol 60:1181, 2001) offered the expectation that this would provide the basis for developing compounds to enable the study of the roles of the endogenous trace amines. Thus, the present work was undertaken

to examine the pharmacology of one of these receptors, TAAR1, for clues to developing selective pharmacologic tools.
Methods: Recombinant human and rat TAAR1 receptors were stably expressed in the AV12-664 cell line. Activation of the receptors was determined by the stimulation of cAMP formation.
Results: Studies of the human TAAR1 receptor showed that β-phenylethylamine (β-PEA) had the highest potency (EC_{50} = 105 ± 4.5 nM) of any of the putative endogenous trace amines. A synthetic derivative, 2-Cl-β-PEA, had the highest potency of any agonist tested (EC_{50} = 33 ± 4 nM). This compound also exhibited relative selectivity for hTAAR1 compared to other tested monoaminergic receptors (e.g., serotonergic and dopaminergic). Screening of a broad series of known nonselective monoaminergic receptor antagonists that cross-react with multiple classical monoaminergic receptors yielded no high affinity compounds for hTAAR1. Comparison of human and rat TAARs revealed some similarities between the two receptors, e.g., β-PEA had an EC_{50} = 209 ± 18 nM at the rat receptor. However, some agonists having relatively low potency at hTAAR1 had high potency at rTAAR1. This is exemplified by 3-iodothyronamine (EC_{50} rat = 22.4 ± 1.8 nM; human = 1510 ± 230 nM). Likewise, certain antagonist molecules (especially certain β–adrenergic antagonists) had significant potency to inhibit rTAAR1 but essentially no effect on hTAAR1. For example, alprenolol gave a Ki for rat = 450 ± 156 nM, while at the human receptor it was greater than 10,000 nM. Propranolol, another β–adrenergic antagonist, showed a similar differential potency between the two receptors.
Discussion: Overall, the pharmacologic profile of hTAAR1 distinguishes itself from that of the classical (serotonin, dopamine, norepinephrine, and histamine) monoamine receptor subtypes. This suggests the possibility of ultimately developing TAAR1 selective compounds to aid in the in vivo study of this receptor. However, the interpretation of the pharmacologic profile of TAAR1 was confounded by the finding of significant pharmacologic differences between the human and rat versions of the receptor. Comparison of their sequences revealed over 20 amino acid differences within the putative transmembrane domains of the two receptors, i.e., the regions most likely to affect drug binding. It, therefore, appears that the development of TAAR1-selective pharmacologic tools will be a significant challenge unless a common pharmacophore can be developed to account for the species differences within the ligand recognition domain of the receptor.

161. Methylphenidate in Pervasive Developmental Disorders: An Analysis of Secondary Measures

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Methylphenidate has previously been shown to improve hyperactivity in about half of treated children with pervasive developmental disorders (PDD) and significant hyperactive-inattentive symptoms. We present secondary analyses to better define the scope of effects of methylphenidate on symptoms that define attention-deficit/hyperactivity disorder (ADHD) and oppositional defiant disorder (ODD), as well as the core autistic symptom domain of repetitive behavior.
Methods: Sixty-six children (mean age 7.5 years) with autistic disorder, Asperger's disorder, and PDD not otherwise specified were randomized to varying sequences of placebo and three different doses of methylphenidate during a 4-week blinded, crossover study. Methylphenidate doses used approximated 0.125, 0.25, and 0.5 mg/kg/dose twice daily with an additional half-dose in the late afternoon. Outcome measures included the SNAP-IV (ADHD and ODD scales) and the Children's Yale-Brown Obsessive Compulsive Scales for PDD.
Results: Methylphenidate was associated with significant improvement that was most evident at the 0.25 and 0.5 mg/kg doses. Hyperactivity and impulsivity improved more than inattention. There were not significant effects on ODD or stereotyped and repetitive behavior.
Discussion: Convergent evidence from different assessments and raters confirms methylphenidate's efficacy in relieving ADHD symptoms in some children with PDD. Optimal dose analyses suggested significant inter-individual variability in dose-response.

162. Supportive-Expressive Group Therapy and Survival in Patients with Metastatic Breast Cancer: A Randomized Clinical Intervention Trial

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Background: This study was designed to determine the effect of intensive group therapy on survival time of women with metastatic breast cancer. Findings regarding the question of whether such psychosocial support affects survival have been mixed.
Methods: Study participants were 125 women with confirmed metastatic (n = 122) or locally recurrent (n = 3) breast cancer. Women randomly assigned to the group therapy condition (n = 64) received educational materials plus supportive-expressive group therapy involving weekly 90-minute sessions led by two experienced coleaders for one to 12.5 years. Women assigned to the control condition (n = 61) received only educational materials.
Results: Fourteen years after the first participant was randomized, overall mortality in the sample was 86%, and median survival time was 32.83 months. No overall statistically significant effect of treatment on survival was found for the treatment (median = 30.74 months) compared to control (median = 33.32 months) samples (hazard ratio = 0.93 percent confidence interval, 0.62 to 1.40; p = 0.73) using an intent-to-treat design. However, in exploratory moderator tests there was a significant moderator effect for estrogen receptor (ER) status (p = 0.002) suggesting that ER negative participants randomized to treatment survived longer than controls, while the ER positive participants showed no treatment effect.
Discussion: The earlier finding that longer survival was associated with supportive-expressive group therapy was replicated only among the ER negative women. Thus possible psychosocial effects on survival may be salient to women who are more refractory to current hormonal treatments.

163. Molecular Mechanisms Underlying Stress-Regulated Adrenergic Function

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Background: Environmental, physiological or environmental stress initiates a sequelae of responses starting with cortisol and epinephrine (EPI) release into the circulation. The stress hormone epinephrine is regulated in part through its biosynthesis by phenylethanolamine N-methyltransferase (PNMT). As a regulator of epinephrine production, PNMT serves as a marker for adrenergic function. Hormones and neural stimuli have been shown to regulate PNMT in part via the PNMT gene. Immobilization (IMMO) stress in rats has been used to demonstrate that stress, which activates hormonal and neuronal regulatory pathways stimulates PNMT gene transcription.
Methods: Male Sprague Dawley rats, immobilized for 30 or 120 min, once or daily up to 7 days, were sacrificed immediately or up to 24 h

later and adrenal glands or adrenal medulla collected according to procedures consistent with the NIH Guide for Care and Use of Laboratory Animals as approved by the McLean Hospital IACUC. Adrenal corticosterone (CORT) was determined by RIA and adrenal dopamine (DA), dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE) and epinephrine (EPI) measured by HPLC. PNMT and transcription factor mRNA were assessed by ribonuclease protection assay or radioactive RT-PCR using total RNA from adrenal glands/medulla. Chemiluminescent western analysis was used to examine PNMT and transcription factor (TF) protein in nuclear or cytosolic fractions isolated from the medulla while gel mobility shift assays (GMSAs) were use to examine TF/DNA binding element interaction.

Results: Single and multiple IMMO rapidly and transiently induces CORT, providing sufficient levels to activate glucocorticoids receptors (GR) with no apparent desensitization through 7 days. While DA is elevated, EPI remains relatively unaltered by IMMO. A rise in the mRNA and protein of the PNMT transcriptional activators Egr-1 and Sp1 prior to or coincident with PNMT mRNA induction occurs. IMMO also affects Egr-1 and Sp1 phosphorylation permitting their greater interaction with cognate DNA binding elements in the PNMT promoter. Changes in PNMT protein are also marked. However, PNMT and TF protein changes are not identical to mRNA in all cases.

Discussion: EPI, an important component of the stress response and activator of physiological processes countering stress, is a critical constituent in stress-related disorders, such as cardiac disease, behavioral illnesses and immune dysfunction. EPI is regulated in part via its biosynthesis by PNMT through control of PNMT gene transcription. Findings reported here provide the first evidence that transcriptional regulation may be important in orchestrating adrenergic responses to stress. IMMO stress markedly increases PNMT mRNA expression, preceded by a rapid and transient elevation of CORT and thereby activation of GRs and at least two additional PNMT gene transcriptional regulators, Egr-1 and Sp1. Downstream of PNMT gene stimulation, corresponding changes in PNMT protein are evident. Since adrenal medullary epinephrine remains unchanged and elevated circulating EPI is sustained in response to stress, biosynthesis of EPI appears to replenish adrenal medullary pools and sustain stress-elicited, elevated levels in the blood stream to support physiological responses necessary to invoke adaptive changes to counter the adverse effects of stress. Differences in PNMT and TF mRNA and protein, and in the case of the latter, phosphorylation, indicate that post-transcriptional controls also contribute to stress-regulated PNMT and thus, epinephrine, expression.

164. Lack of Physiological Regulation of the Expression of Co-Chaperones of the Glucocorticoid Receptor in Peripartum Depression

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Sponsor: Past Travel Awardee, Young Investigator, Memorial, 2005

Background: Major depression during pregnancy and the postpartum period is a major women's health concern. In primates, including humans, but not other species, pregnancy induces dramatic changes in the function of the hypothalamic-pituitary-adrenal (HPA) axis due to placental production of corticotropin releasing hormone (CRH). These changes are similar to those observed in depression and lead to substantially elevated maternal levels of circulating cortisol throughout gestation. Several regulatory mechanisms appear to limit the activity of cortisol during pregnancy, including increases in cortisol binding globuline and an increase in glucocorticoid receptor (GR)-resistance, which is observed in up to 80% of pregnant women. We propose that one potential mechanism altering GR sensitivity in pregnancy could be changes in the expression profile of co-chaperones of the GR, which are responsive to sex-steroids and have been shown to regulate GR signaling. Dysfunction of these physiological regulatory mechanisms may put women at greater risk for depression in the peripartum period, by impairing the buffering of pregnancy-associated HPA-axis hyperactivity.

Methods: To test this hypothesis, we first measured mRNA expression of 10 co-chaperones of the GR in peripheral-blood monocytes (PBMCs) using quantitative RT-PCR during pregnancy in euthymic women (time points: pre-conception, first, second and third trimester and 0-8 and more than 12 weeks postpartum in a total of 127 euthymic women). We then compared chaperone expression profiles of 18 non-medicated depressed women (mean BDI = 27.0 (6.4)) and 26 non-medicated euthymic women (mean BDI = 6.5 (4.3)) in the second trimester of pregnancy. mRNA expression of the co-chaperones FKBP5, FKBP4 and STIP1 were also measure in PBMCs through-out gestation in female Long-Evans rats at E5, E10, E15, E20 and 21 days postpartum.

Results: We observed a significant up-regulation of 6 of the investigated co-chaperones (FKBP5, FKBP4, STIP1, BAG1, PPID and NCOA1 ($p < 0.001$ for all genes)) during human pregnancy that returned to preconception levels after 12 weeks of gestation. FKBP5 mRNA levels were upregulated the most, with second trimester levels 5 fold higher than preconception levels. The observed pattern of chaperone expression regulation would favor the reported GR resistance in pregnancy. These regulatory mechanisms may be exclusive to species with placental CRH expression that leads to pregnancy-associated HPA-axis hyperactivity, as co-chaperone expression did not seem to be regulated through-out rat gestation, a species lacking placental CRH. When comparing chaperone expression in depressed vs. non-depressed women in the second trimester, the previously observed pregnancy-related up-regulation of GR chaperones was not seen in depressed women. For FKBP5 for example, the mean up-regulation compared to a preconception control group in the non-depressed second trimester women was 4.7 vs. 1.2 in the depressed group ($p = 0.001$).

Discussion: The expression of several co-chaperones of the GR is up-regulated in human pregnancy and the early postpartum period and the pattern of this regulation would favor GR-resistance. The regulation of these chaperones via reported sex-steroid responsive element may thus constitute a novel molecular mechanism responsible for pregnancy-associated GR resistance. This regulatory mechanism is dramatically impaired in depressed pregnant women, so that the lack of a physiological regulation of GR chaperone expression during pregnancy may predispose to depressive symptoms by altering GR sensitivity and thus the capacity to buffer pregnancy-associated HPA-axis hyperactivity. Supported by MH076024-01 and Pfizer Scholar Award in Clinical Psychiatry