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Poster Session I - Monday

1. Statistical and Pharmacoeconomic Issues for Annual Alzheimer Screening

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Sponsor: Jared Tinklenberg

Background: A major problem in the field of Alzheimer's disease is the clinical recognition of cases early in the course of the disease, both because treatments may be more effective when started soon and an important research issue is the study of the initial phase of the disease. This study sought to define issues and calculate estimates of the consequences of annual screening for dementia. First, the continuum of pathology which is the target of the screen must be defined, as well as the level of pathology which will be screened. This leads to selecting a "gold standard" for comparison, and its fallibility must also be considered. Then a screening test must be selected with assessed sensitivity (Se) and specificity (Sp). Additional issues considered in the evaluation of such screening tests are the annual incidence in the population (I, doubling every 5 years for Alzheimer's disease, as shown by previous studies), the fixed cost of the test ($T), the cost of false positives ($C ~ $500 for unnecessary evaluation), the cost of false negatives (considered negligible in this discussion), and the benefit of true positives ($B ~ range from $50,000 at 50 y/o to $0 at 100 y/o, partially using data that suggest that early initiation of medication treatment can delay nursing home placement, with a greater impact in younger individuals).
Methods: From these estimated parameters, the cost-worthiness of a screening test was estimated using the equation: $W = ($B x I x Se) - ($C x (1-I) x (1-Sp)) - $T Solutions to this equation were analyzed systematically using various Se and Sp values.
Results: A test with a Se and Sp of 0.8 (like the Mini-Mental State Exam) would reach cost-worthiness at $40 annual administration cost beginning at 75 y/o. A test with Se and Sp of 0.9 would be worth giving annually beginning at 65 y/o if it cost only $10 to administer. Values were calculate for APOE genotype (using our recently published estimate of age-specific incidence by genotype), with a test having Se and Sp = 0.9. For an e4/4 individual a test would be worth $30 for annual screening beginning at 50 y/o, for an e3/4, beginning at 63 y/o and for an e3/3, beginning at 74 y/o.
Discussion: Currently available Alzheimer screening tests were calculated to be cost-effective for annual use, depending on the age and genotype of the individual to be screened. Further improvements of tests will decrease the age at which annual screening should be started. Screening tests can help identify patients at a relatively earlier point in the disease progression, so that interventions can be started sooner, with potential financial benefit for society. Earlier recognition of patients can assist in the study of Mild Cognitive Impairment as a condition prodromal to Alzheimer's disease.

2. Alterations in Brain CRF and SRIF in a Transgenic Mouse Model of Alzheimer's Disease (Tg2576) Following Treatment with a High Fat Diet

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Background: Cortical levels of both somatostatin (SRIF) and corticotropin-releasing factor (CRF) are significantly decreased in postmortem Alzheimer's disease (AD) tissue. A high fat diet and high cholesterol have recently been established as risk factors for human AD and have also been shown to increase beta amyloid production in transgenic mice with APP mutations.
Methods: Using male Tg2576 mice transgenic for the Swedish mutation in human amyloid precursor protein (APP) and age-matched, wild-type controls, we examined concentrations of CRF and SRIF in 14 brain regions at 18 months. Half the cohort was maintained on a high fat diet from 7 months causing an increase of amyloid plaque formation in the entorhinal cortex relative to transgenic mice on a normal lab chow diet.
Results: The trangenic mice (both high fat (AF) and regular diet groups (AR)) had increased locomotor activity from 4 months (before diet alterations) when compared to wild type mice ($p = 0.01$) and while this relative activity increase was maintained throughout the 18 month period, statistical significance was not maintained. We did not observe a spatial memory deficit in these mice, using a probe trial in the Morris water maze 24 hours after training. However, at 18 months over 80% of AF subjects and 56% of AR subjects were unable to learn the platform location despite completing up to 30 training trials with a visible platform in each session. On average 80 % of control subjects (regular diet controls (CR), Fat diet controls (CF)) were able to learn the task within 10 trials. Altered CRF concentrations were observed in several cortical and sub cortical brain regions in the APP mice relative to controls. In the caudate nucleus the CF group had higher CRF levels than the AF group ($p=0.05$ posterior caudate, $p = 0.06$ anterior caudate) and the CR group ($p = 0.01$ posterior, $p = 0.04$ anterior). Significant increases in CRF were also seen in the frontal cortex where CF levels were increased significantly from AF levels ($p = 0.001$) and from CR levels ($p = 0.04$). AF levels were decreased compared AR levels ($p = 0.009$) SRIF levels were also altered in several of the regions assayed. In the hypothalamus the CF group had higher SRIF levels relative to both the AR ($p = 0.021$) and the AF group ($p = 0.03$). Similar results were seen in the cerebellum and ventral brain stem. In the nucleus accumbens the CR group had increased levels of SRIF when compared to the AF group ($p = 0.01$) and the AR group ($p = 0.03$). In the anterior septum the SRIF was also increased in the CR group relative to the AF group ($p = 0.008$), the AR group ($p = 0.02$) and the CF group (0.01)
Discussion: These data indicate that the response of wild-type mice to a high fat diet increases CRF content in caudate and frontal cortex relative to wild-type mice on a normal diet. With the exception of the hypothalamus and the brain stem, the high fat diet caused a decrease in SRIF in the wild-type animals compared to those on a normal diet. The presence of the APP transgene largely prevented these dietary effects on CRF or SRIF in these regions. Thus a diet that increases amyloid plaque load in Tg2576 mice does not reproduce or accelerate the CRF and SRIF deficits that are seen in clinical AD.

3. Participation in Alzheimer's Disease Clinical Trials: A Focus on African American Caregivers

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Sponsor: Past Travel Awardee, NIMH, 2003

Background: The under-representation of racial and ethnic minority elders remains a problem in Alzheimer's disease (AD) research. Although current literature highlights numerous barriers that may hinder the participation of ethnic minority elders in research, there is little known about the "actual" factors behind the decision-making process of African Americans to participate in AD research. This report describes factors that are critical in African American AD caregivers' decision-making process to participate in AD clinical trials.
Methods: 6 focus groups (N=37) consisting of four to seven African Americans (55 years of age and older) were conducted. A seven-item focus group discussion guide was utilized by a trained moderator. All sessions were tape-recorded and transcribed. Content analysis was used to analyze narrative data and identify emerging themes.

Results: All participants self-identified themselves as African Americans. 50% of the focus groups were female caregivers. Analysis of transcripts revealed recurring focus group themes that may influence the decision-making process. Participants appeared to lack the knowledge of Food and Drug Administration (FDA)-approved medications indicated in the treatment of AD. They expressed self-concerns about forgetting and the difficulty in understanding normal aging versus memory loss associated with AD. Participants associated research with a variety of meanings. Lack of trust of physicians appeared to be associated with hesitance to participate in medication-intervention trials. Input of primary care physicians and family members were deemed important in the decision-making process. Prior knowledge of the investigator appeared to be associated with willingness to participate. More detailed results will be presented on the similarities and differences between female and male caregiver focus groups.
Discussion: These focus groups have provided insights on African American AD caregivers' beliefs about Alzheimer's disease, research, and the decision-making process to participate in AD clinical trials. Although the focus group themes highlight the effect of distrust in the decision-making process, our research does not confirm the other common barriers cited in the literature. In the future, the focus group data will be utilized to develop and test strategies to enhance the recruitment and retention of African Americans into AD clinical trials.
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4. Guanfacine Augmentation of Donepezil In The Treatment of Alzheimer's Disease

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Sponsor: Kenneth Davis

Background: Cholinomimetic therapies for AD (Alzheimer's disease) have met with only limited success. Although a sub-group of patients have a modest, albeit clinically significant improvement with cholinesterase inhibitors, this is hardly as robust a treatment as L-dopa for Parkinson's disease or even haloperidol for schizophrenia. Clearly, one problem with a purely cholinergic approach to the psychotherapeutics of AD is that AD is not simply a cholinergic deficit. For example, a noradrenergic deficit is undoubted in a subgroup of patients. Loss of locus ceruleus neurons is present in AD (Mann et al, 1982), is evident among subjects with the greatest neocortical plaque formation (Tomlinson et, 1981) and is correlated with severity of dementia (Bondareff et al, 1981). Guanfacine, a selective alpha-2a agonist has been demonstrated to increase noradrenergic neurotransmission in the frontal cortex and improve cognitive performance in a number of paradigms. Therefore, our aim was to determine if the co-administration of the selective alpha-2a agonist, guanfacine, with donepezil is clinically more effective in treating cognitive symptoms of AD than administration of donepezil alone.
Methods: The design is a double blind, placebo controlled, parallel designed investigation comparing treatment with donepezil alone to treatment with donepezil in combination with guanfacine at a dose of 2 mg per day. The study was carried out over 21 weeks and was divided into four phases: 1) screening and baseline, 2) titration (The dose of guanfacine was titrated up to 1 mg twice a day over a period of four weeks in subjects receiving active treatment), 3) treatment (the dose of guanfacine remained at 1 mg twice daily in subjects receiving active treatment for a period of fourteen weeks), 4) taper down (starting week nineteen the dose of guanfacine was reduced to 1.0 mg once a day, then discontinued on week 20, then subjects continued only on donepezil for week 21). Efficacy measures including ADAS (Alzheimer's Disease Assessment Scale), CIBIC-Plus, NPI (Neuropsychiatric Inventor), CAS (Caregiver Activity Scale), and PDS (Progressive Deterioration Scale) were performed at baseline, week 4, week 10, week 18 and week 21. In addition, subjects were seen weekly for safety monitoring including blood pressure and assessment of adverse effects
Results: A total of 39 subjects were randomized to double blind treatment. This study has completed and all data has been collected, verified and entered. Analyses of the data are currently underway and will be presented.
Discussion: The implications of the results of this study to the treatment of Alzheimer's Disease will be dicussed.

5. Anxiolytic Actions of an Estrogen Receptor Beta Agonist in Aged Female Rats

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Sponsor: Sheryl Beck

Background: The actions of estrogen on the brain are mediated by two estrogen receptor (ER) subtypes, ERalpha and ERbeta. Whereas ERalpha plays a predominant role in controlling reproductive function, this does not appear to be the case for ERbeta. Recent studies in rodents have indicated a potential role for ERbeta in mediating the anxiolytic actions of estrogen. In behavioral studies, treatment with a selective ER beta agonist, diarylpropionitrile (DPN), increased anxiolytic behaviors and decreased anxiogenic behaviors (Lund et al., Endocrinology 146:797, 2005; Walfe and Frye, Neuro-psychopharmacol. 10:1288, 2005). Consistent with these findings are the results of studies demonstrating greater anxiety-related behaviors in female ERbeta knockout mice (Krezel et al., PNAS, 98:12278, 2001; Imwalle et al., Phys. Behav. 84:157, 2005), and a corresponding loss of estrogen's anxiolytic actions in ERbeta knockouts (Rocha et al., Psychopharmacol. 179:637, 2005). Given that changes in emotional behaviors have been reported in both aging humans and rats; in this study we tested the hypothesis that treatment with the selective ERbeta agonist, DPN, could alleviate age-related changes in anxiety or fear-related behaviors.
Methods: Initially, intact male and female Sprague-Dawley rats were examined for changes in open-field behaviors across several ages from 4 months to 24 months of age. Subsequently, we treated aged (18-22 mos. old) intact female rats with DPN (1 mg/kg BW, once daily for 4 days) or vehicle and examined their behavior in the open field and elevated plus maze following the last injection.
Results: We detected age-related increases in anxiogenic behaviors (grooming, fecal boli), and decreases in anxiolytic-like behaviors (activity, investigation, rearing, center squares) that were all significantly changed in both sexes by 17 months of age. Consistent with this were age-related increases in plasma corticosterone levels. In the elevated plus maze, DPN treatment of aged female rats resulted in significant increases in anxiolytic behaviors such as open arm entries, time spent on the open arm, and head dips. DPN treatment significantly reduced anxiogenic behaviors such as grooming and fecal boli. Similarly, in the open field, DPN treatment significantly increased anxiolytic behaviors such as rearing and decreased anxiogenic behaviors such as fecal boli. Increases in plasma corticosterone levels in response to the stress of the behavioral testing apparatus were correspondingly decreased by DPN.
Discussion: Thus, these studies further demonstrate that ERbeta agonists can have anxiolytic properties and raise the possibility that ERbeta agonists can be used as an effective anxiolytic agent in aging populations.
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6. The Neuroanatomical Patterns Associated With Neuropsychiatric Symptoms In Older Adults With And Without Cognitive Impairment: An MRI Study

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Sponsor: Gary Small

Background: We examined the MRI brain structural changes associated with neuropsychiatric symptoms in a cohort of older adults with and without cognitive impairment.
Methods: Subjects were recruited to participate in a multi-center collaborative study of subcortical ischemic vascular disease (SIVD). They received a comprehensive neurobehavioral evaluation of cognitive impairment and behavioral symptoms. Subject recruitment was targeted for six groups defined by three levels of cognition (normal or CDR=0; impaired or CDR=0.5; demented or CDR>=1) by the presence/absence of lacunes. Subjects underwent an MRI and classification of the presence of subcortical lacunar infarcts was based on standard quantitative procedures and the consensus diagnosis. MRI volumetric measures of brain structures included volumes of lacunar infarcts in specific subcortical structures, volume of white matter signal hyperintensities (WMSH), volume of cortical gray (cGM) and white matter (WM), and CSF, and total hippocampal volume (HV). Neuropsychiatric assessments were done for each of the DSM-III-R criteria for depression, anhedonia, anergia, and apathy based on the symptom duration and severity. Logistic regression analyses were used to evaluate associations between neuropsychiatric symptoms and MRI variables and cognitive status, adjusted for age, gender, and race.
Results: A total of 270 subjects (160 (59%) men; 214 (79%) Caucasian; mean age 74.4 years; range 52.8- 94.7 years) were evaluated at baseline with neuropsychiatric assessment and quantitative MRI. The distribution of cognitive status included subjects: cognitively intact (103; 38%), cognitively impaired (74; 27%), or with dementia (93; 34%); 112 subjects (41%) had lacunes on MRI. The prevalence of clinically significant depression was 18.1%; anhedonia- 12.7%; anergia- 26.3%, and apathy- 18.2%. There was only up to 30% overlap in these symptoms among subjects. Subjects with neuropsychiatric symptoms were more likely to be cognitively impaired or demented, have lacunes, had larger total lacunar volume, as well as lacunar volume in the putamen, thalamus, white matter, and higher WMSH volume. Subjects with neuropsychiatric symptoms had smaller volumes of cGM, and WM. In the multivariate analysis, higher total lacunar volume was associated with the presence of depression, anhedonia, and apathy, but not anergia. Lower white matter volume was associated with anergia and apathy, and higher volume of white matter signal hyperintensities was associated with anergia. Age was inversely related to the presence of anergia and apathy.
Discussion: We found different neuroanatomical patterns on MRI changes associated with neuropsychiatric symptoms of depression, apathy, anhedonia, and anergia in older subjects with and without cognitive impairment. Our findings provide new understanding of the neuroanatomical characteristics of late-life neuropsychiatric symptoms, and have broad implications for the neurobiology of behavior supporting further investigation of late-life behavioral endophenotypes using neuroimaging.

7. Deficit in the GABAergic Neuroactive Steroid Allopregnanolone in Alzheimer's Dementia and Relevance to Neuropathological Disease Stage: Investigations in Temporal Cortex

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Background: Recent evidence suggests a role for neuroactive steroids in neurodegenerative disorders. The GABAergic neuroactive steroid allopregnanolone (ALLO) is significantly decreased in Niemann-Pick type C mice, and neonatal ALLO administration delays neurological symptom onset and doubles lifespan in these animals (Griffin et al 2004). We recently determined that ALLO levels are also decreased in prefrontal cortex in male subjects with Alzheimer's disease compared to cognitively intact male control subjects, and that ALLO levels are negatively correlated with pathological disease stage (Braak and Braak staging method), n=14-15 per group (Trost et al 2005). Furthermore, ALLO impacts myelination (Ghoumari et al 2003), and an age-related dysregulation in myelination may contribute to the pathophysiology of Alzheimer's disease (Bartzokis 2003, Benes 2004). ALLO also increases proliferation of rodent and human neural progenitor cells (Wang et al 2005). ALLO may thus play a critical role in the pathophysiology of neurodegenerative disorders including Alzheimer's disease. We therefore investigated ALLO levels in postmortem temporal cortex tissue from patients with Alzheimer's disease and cognitively intact control subjects.
Methods: Temporal cortex postmortem tissue from the Joseph and Kathleen Bryan Alzheimer's Disease Research Center at Duke University was analyzed for ALLO by highly sensitive and specific gas chromatography/mass spectrometry, preceded by high performance liquid chromatography. ALLO levels were determined in temporal cortex from 40 subjects with Alzheimer's disease (23 females, 17 males) and 41 cognitively intact control subjects (20 females, 21 males). Subject age and postmortem interval (PMI) did not differ between groups.
Results: ALLO levels were significantly decreased in temporal cortex in patients with Alzheimer's disease (median 2.68 ng/g, n=40) compared to cognitively intact control subjects (median 5.64 ng/g, n=41), Mann-Whitney p=0.0002. ALLO levels were negatively correlated with neuropathological disease stage (Braak and Braak), Spearman r = -0.39, p=0.0005. ALLO levels were not related to PMI.
Discussion: There is an ALLO deficit in temporal cortex postmortem tissue in subjects with Alzheimer's disease compared to cognitively intact control subjects, and lower ALLO levels are correlated with greater pathological disease stage severity. These data are consistent with our prior findings in prefrontal cortex. Neuroactive steroids may play a role in Alzheimer's disease and represent potential novel treatment targets.

8. The Allosteric Potentiation of Nicotinic Acetylcholine Receptors by Galantamine Ameliorates the Cognitive Dysfunction in the Amyloid-Treated Mice: Involvement of Dopaminergic Systems

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Sponsor: Edward Domino

Background: Galantamine, a drug for Alzheimer's disease (AD), is a novel cholinergic agent with a dual mode of action, which inhibits acetylcholinesterase and allosterically modulates nicotinic acetylcholine receptors. Galantamine has been demonstrated to have potential cognitive-improving effects on AD since the allosteric action of galantamine may be related, in part, to the stimulation of catecholamine neurotransmission in addition to its enhancing effects on cholinergic systems by inhibition of acetylcholinesterase. To confirm such events, in the present study, we investigated whether the galantamine shows the cognitive-improving effects through the allosteric modulation of nicotinic acetylcholine receptors in an animal model of AD.
Methods: Male ICR mice, aged 6 weeks at the beginning of the experiments were used. The mice were anesthetized with ether and were intracerebroventricularlly injected 3 μl of vehicle (distilled water) or 9 nmol/3 μl of amyloidβ $(Aβ)_{25\text{-}35}$. The novel-object recognition and

conditioned fear learning tests were carried out on the day 7-8 and 9-10, respectively, after the injection of $A\beta_{25-35}$. On the day 10 after the injection of $A\beta_{25-35}$, the effects of galantamine on the extracellular level of dopamine (DA) release were determined in the hippocampus of $A\beta_{25-35}$-treated mice by using in vivo microdialysis method. The present study was designed to study in a blind manner. All experiments were performed in accordance with Guidelines for Animal Experiments of Nagoya University Graduate School of Medicine.
Results: Galantamine (3 mg/kg p.o.) increased the extracellular level of DA release significantly in the hippocampus of vehicle- and $A\beta_{25-35}$-treated mice. The effects of galantamine on the extracellular DA release were antagonized by mecamylamine, a nicotinic receptor antagonist. $A\beta_{25-35}$-treated mice, compared with vehicle-treated mice, could not discriminate between new and familiar objects in the novel-object recognition test and exhibited less freezing response in the conditioned fear test, indicating the cognitive dysfunction. Galantamine (3 mg/kg p.o.) improved the cognitive dysfunction induced by intracerebroventricular injection of $A\beta_{25-35}$ in the novel-object recognition and conditioned fear learning tests. These improving-effects of galantamine were prevented by treatment with mecamylamine, SCH-23390, a DA-D_1 receptor antagonist and sulpiride, a DA-D_2 receptor antagonist.
Discussion: This study provides the first in vivo evidence that galantamine augments dopaminergic neurotransmission within the hippocampus through the allosteric activatation of the nicotinic cholinergic receptors. The improving-effects of galantamine on the cognitive dysfunction induced by $A\beta_{25-35}$ injection may be mediated through the activation of, at least in part, dopaminergic systems. Thus, we postulate that galantamine may activate dopaminergic neurotransmission in AD by augmenting the stimulative effects of nicotinic cholinergic receptors. This is supported by the fact that galantamine potentiated the hippocampal DA release in the $A\beta_{25-35}$-treated model of AD. Because the dopaminergic dysfunction has been implicated in the progress of AD and dopaminergic agents may be beneficial in treatment, enhancement of DA release may be one of multiple mechanisms underlying the therapeutic benefit of galantamine.

9. Early-Onset Behavioral and Synaptic Deficits in a Mouse Model of Alzheimer's Disease Are Reversed by Inhibition of Gamma Secretase

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Sponsor: Floyd Bloom

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder in humans as well as in mouse models of AD. There is increasing evidence that neuronal dysfunction occurs prior to the accumulation of beta-amyloid (Abeta) containing plaques. Characterization of the timing and nature of pre-plaque dysfunction is important for understanding the temporal progression of this disease, and to identify pathways and molecular targets for therapeutic intervention. Transgenic mice (Tg2576) overexpressing the Swedish mutation of the human amyloid precursor protein (APP) have been used a model of Alzheimer's disease given that they display biochemical, pathological and behavioural markers consistent with many aspects of Alzheimer's Disease including impaired hippocampal function.
Methods: Progression of neuronal dysfunction in Tg2576 animals has been examined using a number of in vitro and in vivo parameters. Histopathological and morphological assesment has been performed using light microscopy and unbiased stereologic sampling of Golgi-impregnated neurons. Functional assesment of Tg2576 hippocampal slice preparations were performed using standard LTP induction protocols. The first conditioning stimulus train was applied to the slice using high frequency bursts (200 Hz) of ten pulses, repeated every two seconds for ten cycles. Second and third conditioning trains were subsequently delivered. After delivery of the third conditioning train, normal test stimulus pulses were continued for one hour. Behavioral assessment of Tg2576 animals was made at various ages using a contextual fear conditoning paradigm in operant chambers. Freezing scores for each animal were converted to percent freezing for each portion of the test. Memory for the context (Contextual memory) for each animal was obtained by subtracting the percent freezing in the novel condition (a measure of basal activity) from that observed in the context. The effect of age and drug treatment on contextual memory were measured.
Results: Measuring spine densities in the molecular layer of the dentate gyrus using unbiased stereologic sampling of Golgi-impregnated neurons, demonstrated a significant reduction of spine densities at 4 months of age in Tg2576 animals. The deficits in synaptic connectivity observed at 4 months of age were closely associated with deficits in hippocampal LTP and the first evidence of impaired hippocampal learning in contextual fear conditioning. At this age, animals showed no evidence of amyloid deposits or any measurable rise of insoluble Abeta42. Acute single dose administration, three hours prior to training, with 100mg/kg (po) of the gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-t-butylester (DAPT), acutely reduced brain concentrations of Abeta and resulted in a significant attenuation of the observed memory impairment in 4 month old Tg2576 mice.
Discussion: Our data show a relationship between reduced spine density, LTP deficits in the hippocampus and impaired memory and suggest this is the earliest evidence of neuronal dysfunction in the Tg2576 mouse model of AD. Furthermore, our data are supportive of a direct role for Abeta in the impairment of memory and suggest that acute treatment with GSIs may provide acute improvement in cognitive function in addition to the reported longer term disease modifying effects of chronic amyloid lowering.

10. Frontal, Parietal, and Callosal Degradation in Aging: A Quantitative DTI Fiber Tracking Study

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Background: The effects of normal adult aging are subtle, accrue insidiously, and can be elusive to detection with conventional macrostructural neuroimaging techniques. In vivo characterization of white matter features is possible with diffusion tensor imaging (DTI), an MR imaging method sensitive to the detection of white matter's linear structure. By taking advantage of the molecular diffusion of water, the primary constituent of the brain's composition, DTI can detect the microenvironment of white matter and provide assessment of physical characteristics of white matter fibers, which vary widely in length, diameter, and myelination and by region. Postmortem studies report a predilection of loss of thin, unmyelinated fibers, which are in greatest abundance in the frontal lobes and genu of the corpus callosum. In vivo DTI studies based on a regional samples support the anterior-to-posterior gradient of white matter disruption but, to date, have not taken advantage of DTI's ability to depict white matter systems through fiber tracking, expressing voxel-to-voxel connectivity between different brain regions. Fiber tracking yields compelling visual displays and can be used in quantification of fiber integrity.
Methods: We implemented a DTI fiber tracking routine, based on an extraction of the Principal Diffuse Direction (PDD) of the tensor field. To control for morphological differences, men and women and young and old subjects all were placed in a standardized coordinate system using rigid, followed by nonlinear warping based on volumetric MR images. The final nonlinear function for each subject was then used to place one's own structural, fractional anisotropy (FA), and apparent diffusion coefficient (ADC) images into the standardized

coordinate system with a single resampling. A software routine was developed to identify the fibers that reached both left and right forceps, the fibers that reached just one forcep, the fibers that doubled back on themselves, and the fibers that reached one forcep but then coursed posteriorly from the genu or anteriorly from the splenium on the other side. An interactive program allowed the "clipping" of these latter errant fibers at the point they coursed away from the forceps-callosal loop. DTI data were collected at 3T in 10 younger (22-37 years old) and 10 older (65-79 years) healthy, highly educated men and women. Fiber tracking focused on the frontal and posterior forceps, the bilateral extents being connected by the genu anteriorly and splenium posteriorly.
Results: Older subjects had lower FA (p=.0001), higher ADC (p=.0009), and fewer (p=.02) and shorter (p=.002) identified fibers. Group-by-region interactions indicated disproportionately lower FA (p=.0001), shorter fiber length (p=.032), and a trend for fewer fibers identified (p<.10) in the older relative to the younger group, in frontal relative to posterior regions.
Discussion: These results provide confirmation of the selective vulnerability of frontal white matter systems to normal aging and validation of this quantitative fiber tracking approach. The observed signs of aging selective to prefrontal circuitry likely contribute substantially to decline in executive functioning, a typical concomitant of normal aging, and pose a vulnerability to insult by neuropsychiatric diseases, which commonly target frontal systems. Support: National Institute on Aging (AG17919) and National Institute on Alcohol Abuse and Alcoholism (AA12388, AA12999)

11. Subjective Cognitive Impairment: The Pre-Mild Cognitive Impairment Stage of Brain Degeneration: Longitudinal Outcome After a Mean of 7 Years Follow-Up

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Background: Mild cognitive impairment (MCI), is now a recognized entity.[1-3] In 1982, we published a description of this MCI stage as stage 3 on the Global Deterioration Scale (GDS).[1] In the GDS, we described two earlier stages, i.e., a stage in which older persons are free of subjective or objective impairments (non-SCI, GDS stage 1), and a stage in which older persons have subjective cognitive impairment only (SCI, GDS stage 2). Using a longitudinal data set, outcomes were studied in subjects categorized at baseline into the two stages of normal aging.
Methods: Healthy persons evaluated from 1/1/1984 to 12/31/97 were studied. Follow-up (F/U) data obtained until 12/31/01 was utilized. Subjects (N = 250) were followed, 53 non-SCI, and 197 SCI. Time to F/U was: (1) time to progression to MCI or dementia; or, if no progression, (2) time to the last F/U prior to 2002. The F/U interval was 7.0±3.6 years. There were significant differences between non-SCI and SCI groups respectively, in age (mean = 64.0 and 67.4 yrs, $p < 0.05$) and MMSE (mean = 29.6 and 28.9, $p < 0.001$). There were no significant differences in F/U interval (6.8 and 7.1 yrs), gender (56.6 % and 66.0 % female), or education (16.1 and 15.7 yrs).
Results: Of non-SCI subjects, 9 of 53 progressed; of SCI subjects, 112 of 197 progressed (outcome differentials were significant, Fisher's exact test, $p < 0.0001$). Mean decline time for non-SCI subjects (3200 days) was also greater than the SCI subjects (2045 days) (Savage two-sample test for event time, $p = 0.0007$). Because of significant group differences, a pair wise matching procedure was used to select two groups matched by age and MMSE scores, as well as gender. For the resulting 31 matched pairs, there were no significant differences in age, MMSE or education; significant differences remained in terms of both outcome and time to progression. For the non-SCI group, 8 subjects progressed, 23 did not; for the SCI group, 18 subjects progressed, 13 did not (McNemar's test, $p = 0.012$). The mean time to deterioration in the 8 non-SCI subjects was 3239 days, the mean time for the 18 SCI subjects was 2156 days (Savage two-sample test for event time, $p = 0.037$). The agreement between the original and the paired data using the following logistic model was fitted to each data set: $\log p / 1-p = \beta_0 + \beta_1 * \text{Group} + \beta_2 * \text{Age} + \beta_3 * \text{MMSE} + \beta_4 * \text{Gender} + \beta_5 * \text{Education}$ where p is the probability of progressing. There is a significant group effect for both data sets: odds ratios, 4.8 for the original data set and 5.2 for the paired data set. Therefore, controlling for age, gender, MMSE and education, SCI subjects are ~5 times more likely to decline than non-SCI subjects.
Discussion: Older subjects free of SCI have a more benign prognosis than subjects with SCI. SCI was a pre-MCI stage in a majority of subjects over a 7-year mean interval. Predictors of progression within this SCI stage are being identified as are physiologic differences between non-SCI and SCI subjects.[4, 5] 1. Reisberg, B., Ferris, S.H., de Leon, M.J. et al. The global deterioration scale for assessment of primary degenerative dementia. Am. J. Psychiatry, 1982, 139:1136-9. 2. Flicker, C., Ferris, S.H., Reisberg, B. Mild cognitive impairment in the elderly: predictors of dementia. Neurology, 1991, 41:1006-9. 3. Petersen RC, et al. Early detection of dementia: MCI. Neurology, 2001; 56: 1133-42. 4. Prichep, L.S., Ferris, S.H., Rausch, L., et al., Prediction of cognitive decline in normal elderly using electrophysiological imaging. Neurobiol. Aging, in press. 5. Wolf, O.T., et al., Subjective memory complaints in aging are associated with elevated cortisol levels. Neurobiol. Aging, in press.

12. Effect of Catechol-O-Methyl Transferase val ^{158} Met Polymorphism on Information Processing in the Prefrontal Cortex during Working Memory in the Elderly

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Background: Cognitive abilities including working memory decline with age, but the rate of this decline is variable across individuals. Part of this variance is thought to arise from genetic factors. Recent functional brain imaging studies have shown that elderly subjects show greater cortical activity than younger subjects to maintain proficiency in performing cognitive tasks. This has been suggested to reflect greater cognitive effort requiring recruitment of additional cortical resources for the performance of the task (i.e. cortical inefficiency). Evidence also shows that catechol-O-methyl transferase (COMT), an enzyme that catalyzes the metabolism of catecholamines, accounts for some of the individual variability observed in prefrontal cortical (PFC) function. The purpose of the current study is to explore the effect of the COMT val ^{158} met polymorphism on age related changes in PFC information processing as well as on age-related changed in the functional connectivity of prefrontal regions during working memory.
Methods: Sixteen elderly healthy subjects [5 met/met (2 males, mean age 59.9 ± 4.0), 4 val/met (3 males mean age 59.4 ± 1.9), 7 val/val (4 males, mean age 58.3 ± 4.3)] and sixteen young healthy controls [5 met/met (2 males, mean age 30.6 ± 5.0), 4 val/met (3 males, mean age 37.4 ± 4.6), 7 val/val (4 males, mean age 32.0 ± 8.1)] were studied with BOLD fMRI on a 3T GE scanner while they performed an N-Back working memory task. The two groups were matched for IQ, gender, handedness and genotype.
Results: There was no significant effect of age, genotype or genotype-age interaction on the one-back working memory task performance [$F(5, 26) = 0.64$, $p = 0.67$]. Preliminary analysis of imaging data during the one-back working memory task revealed a main effect of genotype as well as of age; val/val subjects and elderly subjects show greater prefrontal activity (val/val > met carriers: left BA45/46, x -49, y 26, z 21, $Z = 2.68$, $p = 0.004$, $k = 47$; elderly > young: left BA 9, x -49, y 12, z 33, $Z = 2.48$, $p = 0.007$, $k = 23$; right BA9; x 26, y 38, z 31, Z=

1.91, p= 0.03; k=5) relative to the met carriers and younger subjects respectively. Functional connectivity analysis also showed increased functional connectivity between PFC areas in the val/val and elderly subjects relative to the met/met and young subjects respectively. Most interestingly, we observed that the genotype effect (i.e. greater PFC activity and functional connectivity in the val/val group relative to the met/met group) was much more exaggerated in the elderly subjects; the relative difference in prefrontal activity and functional connectivity between the val/val elderly and val/val young subjects was much more pronounced when compared to the relative difference in prefrontal activity and functional connectivity between the met/met elderly and met/met young subjects.
Discussion: These results while confirming the role of COMT genotype and aging on prefrontal cortical efficiency suggest a protective role of carrying the met allele on cognitive aging.

13. Serotonin Modulates Longevity and Global Gene Expression in the Mammalian Brain

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Sponsor: David Lewis

Background: In addition to being implicated in vascular regulation, sleep, locomotion, endocrine regulation, pain, appetite, sex, aggression, cognition and mood regulation, evidence suggest a role for serotonin (5-HT) during aging. Genetic analyses indicate a possible role in longevity for catecholamines in humans and drosophila. In the brain, structural and functional age-related changes in 5-HT receptor levels have been documented by postmortem receptor binding studies, in vivo imaging studies, and neuroendocrine challenges, however, a causative role for serotonergic signaling in aging has not been established in mammalian systems.
Methods: Life-long changes in behavior were investigated in several behavioral paradigms in mice lacking the 5-HT1B receptor and in control littermates. In parallel, gene expression profiles were generated at different ages in the cortex and striatum of WT and KO mice, and analyzed for age-related effects.
Results: 5-HT1B KO mice develop normally into adulthood and present a baseline unchallenged phenotype in young adulthood that is undistinguishable from their control littermates. Throughout adulthood, a panel of behavioral tests revealed a differential aging phenotype in 5-HT1B KO mice, where KO mice develop an early and progressive deficit in motor coordination, consistently with the role of this receptor in locomotion. Maximum and average lifespans were significantly reduced in 5-HT1B KO mice (~-20%). Control studies suggest that the phenotype is mediated by the absence of 5-HT1B in the brain. Comparative studies of gene expression profiles in WT and KO brains with results from our previous human studies indicated a phylogenetic conservation of age-effect in mammalian cortex, and revealed large shifts in the progression of the molecular correlates of aging in 5-HT1B KO mice.
Discussion: The 5-HT system is emerging as a key system for adaptation to stress, as indicated by gene-environment interaction studies. We view aging as the accumulation of a variety of events that act together to create in essence a chronic challenge to the brain. Accordingly, the interaction of the 5-HT system with this challenge may influence age-dependent behavior and molecular events. Here, we present evidence for a novel serotonergic aging phenotype, based on the targeted mutation of the 5-HT1B receptor, a pre-synaptic autoreceptor. In particular, we establish that altering 5-HT signaling through the 5-HT1B receptor induces age-related progressive declines and results in decreased longevity, thus demonstrating a causative role for 5-HT in aging processes. Control experiments suggest that the reduced longevity originates from a central deficiency, while gene expression studies reveal that altering serotonergic signaling induces a global shift of the molecular correlates of aging in the brain.

14. PET Studies of Cholinergic Modulation of Serotonin in Alzheimer Disease (AD)

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Background: Neuropathologic studies have implicated a presynaptic cholinergic deficit in AD. Deficits in other neurotransmitter systems have been reported, as well (e.g. serotonin, dopamine, glutamate). Since neurotransmitter systems function synergistically, it is possible that cognitive and behavioral symptomatology in AD is affected by the failure of acetylcholine to modulate other functionally-linked neurotransmitters and that cholinergic treatments may improve the capacity of acetylcholine to modulate other functionally-linked neurotransmitters. For example, the cholinesterase inhibitors may also improve symptoms of agitation and psychosis in AD that have been putatively linked to alterations in monoamine systems. Thus, the mechanism of action of the cholinesterase inhibitors may involve effects on monoamine systems. Preclinical data have shown that treatment with cholinesterase inhibitors and modulation of presynaptic nicotinic receptors alter concentrations of dopamine and serotonin. The present study extended these preclinical observations to the study of AD patients by evaluating the effects of treatment with the cholinesterase inhibitor and nicotinic receptor modulator, galantamine, on serotonin function.
Methods: To evaluate cholinergic modulation of serotonin, the glucose metabolic response to intravenous administration of the selective serotonin reuptake inhibitor citalopram was measured in patients at baseline and during treatment with the cholinesterase inhibitor and nicotinic receptor modulator, galantamine. Six patients who met the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for probable Alzheimers Disease were enrolled in the study (mean age 74 ±11, 4F/2M, mini-mental status exam score 23 ± 2). Prior to galantamine treatment, the subjects underwent two resting PET studies to measure cerebral glucose metabolism, performed after administration of a saline placebo infusion (Day 1) and after administration of citalopram (40mg, IV, Day 2). The PET scans were repeated twelve weeks after being titrated to the highest dose of galantamine (24mg). PET data acquisition was performed on the GE Advance PET Tomograph 35 minutes after injection of [18F]-2deoxy-2-fluoro-D-glucose. The PET data were analyzed using the data driven voxel-wise analysis method, statistical parametric mapping (SPM99) methods.
Results: At baseline, the AD patients demonstrated a decrease in glucose metabolism in medial frontal gyrus (bilaterally) and parietal (right inferior parietal lobule and left precuneus). Galantamine treatment increased metabolism in the superior frontal gyrus (left, medial frontal gyrus (right) and precuneus (bilaterally). During galantamine treatment, the patients showed greater increases in cortical metabolism after citalopram in the anterior cingulate gyrus (right) precuneus (bilaterally), superior parietal lobule (left, $p \leq 0.001$, uncorrected) as compared with the response observed prior to initiating treatment.
Discussion: These results suggest, consistent with preclinical evidence, a synergistic interaction of cholinergic and serotonergic systems. These data, in addition to the available pre-clinical data, suggest that combined interventions of cholinergic and serotonergic systems may be potentially effective in AD.

15. Stress History and Breast Cancer Recurrence

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Background: Despite widespread public belief that stress can influence the incidence or progression of breast cancer, research findings

have been inconsistent. Overall, tumor biology seems to dominate host resistance factors, stress-mediated or not. However, there is increasing evidence of the adverse health effects of cumulative stressors and the body's failure to adapt to them. This literature is divided, but suggests that major stressors may be associated with elevated risk of breast cancer incidence or relapse. In the present study we sought to test the hypothesis that a history of highly stressful and/or traumatic life events would be associated with more rapid disease progression among women who develop metastatic or recurrent breast cancer.
Methods: Past experiences of traumatic life events were assessed among ninety nine women with metastatic or recurrent breast cancer. A traumatic event assessment was conducted using the event screening question from the posttraumatic stress disorder (PTSD) module of the Structured Clinical Interview for the DSM-IV-TR (SCID; 2002). Thirty percent of the women reported no history of traumatic life events exclusive of their breast cancer diagnosis, while 70% reported one or more events in response to a query asking about their experience of "extremely upsetting" events. Each reported event was judged by two independent raters to determine whether it met DSM-IV-TR PTSD A1 criteria for a traumatic event. Those events that did not meet such criteria were designated "stressful events." Forty-one percent of the women in the sample were judged to have experienced one or more traumatic events; 28.3% of the sample reported only stressful events.
Results: A Kruskal-Wallis test indicated significant overall differences among the three groups in the length of the disease-free interval. Planned comparisons revealed a significantly longer disease-free interval among women who had reported no traumatic or stressful life events (Median = 61 months) compared to those who had experienced one or more stressful or traumatic life events (Combined Median = 32 months). Those women who reported a history of traumatic life events had the shortest disease-free interval (Median = 30 months, Range 0-252 months), followed by women with a history of stressful life events (Median = 43, Range 0-175 months).
Discussion: These results suggest that a history of stressful or traumatic life events may reduce host resistance to tumor growth. These findings are consistent with a possible long-lasting effect of previous life stress on stress response systems such as the hypothalamic-pituitary-adrenal (HPA) axis, consistent with prior animal and human stress research.

16. Benefits of Memantine/Donepezil Treatment on Behavior in Moderate to Severe AD Using Factor-Analytically Derived Subscales

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Background: Memantine, a moderate affinity, uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is approved in the U.S. for the treatment of moderate to severe Alzheimer's disease (AD) and is also available in Europe. To assess the effects of memantine on behavioral disturbances in AD, behavioral outcomes were analyzed based on a previously published trial of memantine in moderate to severe AD patients receiving stable donepezil treatment.
Methods: Neuropsychiatric Inventory (NPI) domains were aggregated into three subscales based on a modification to a previously reported factor analysis that included the sleep and appetite items onto the Mood subscale. Each subscale was calculated as the sum of items that loaded onto a given factor. The subscales were: Mood (Depression, Anxiety, Irritability/Lability, Night-time Behavior, Appetite/Eating Change), Psychosis (Delusion, Hallucination, Agitation/Aggression), and Frontal (Elation/Euphoria, Disinhibition). The efficacy analysis was based on the ITT population, and Last Observation Carried Forward approaches are reported.
Results: Baseline characteristics between the placebo treatment group and memantine treatment group were comparable. The total NPI score was significantly lower for the memantine group as compared to the placebo group at week 24 (P =.002), representing fewer behavioral disturbances and psychiatric symptoms in memantine-treated patients. At week 24 based on the Mood subscale, memantine/donepezil treated patients had levels that were above baseline, whereas placebo/donepezil treated patients had worsened by 1.6 points (P=.002). Levels of psychosis increased less in memantine/donepezil treated patients than placebo/donepezil treated patients (P=.008)
Discussion: These findings suggest that 6-months of memantine treatment in patients receiving stable donepezil significantly reduces behavioral disturbances in patients with moderate to severe AD, with a robust benefit for behaviors associated with mood and psychosis.

17. Is Translational Research Monkey Business: The Journey of a Novel AMPAKINE (CX 717) from Primate to a Human Phase 1B Study with a Cognitive Impairment Model

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Background: One of the major challenges facing the development of novel chemical entities is the selection of a clinical indication and dose. Animal models have been questioned for their relevance for human neuropsychiatry. Better models would facilitate early clinical development. Glutamate is the predominant neurotransmitter in the mammalian brain, accounting for as much as over 75% of all brain communications. Several different subtypes of receptors recognize glutamate; one of these, the AMPA receptor, is the pharmacological target for CX 717. In a recent study one of us (SamD) described a process in the monkey hippocampus that facilitated recognition memory by encoding objects with related features into distinct categories, thereby increasing the capacity to remember stimuli in a delayed match to sample (DMS). Ampakines are positive modulators of AMPA receptors & facilitate behavioral testing performance in rodents. Accordingly we tested the hypothesis that pharmacological manipulation of AMPA receptor function would reverse the deleterious effects of 30-36 hours of sleep deprivation (SD)on DMS. If positive, a human phase 1B trial would follow.
Methods: Rhesus macaques (n=11) were trained in a visual DMS, 150-300 trials per daily session. Stimuli consisted of clip art images. CX717 was administered intravenously 0.3 to 1.5 mg/kg. The SD regimen was 30-36 hours. Sessions were classified: normal alert, CX717, Sleep deprived (SD) and SD+CX717. The translation into humans was a randomized, double blind, 4-way crossover study in 16 volunteers employing a SD/circadian trough effect design. Subjects received a single oral dose of placebo(P), CX 717 100, 300, or 1000 mg. to achieve levels comparable to those effective in primates. Assessments included: Maintainence of Wakefulness (MoW), continuous tracking test(CTT), sustained attention and reaction time (SART), rapid visual information processing (RVIP), and the critical flicker fusion test (CFF).
Results: The effects of CX717 on performance under normal alert conditions, compared to vehicle, revealed improved overall performance by 15%($p<0.001$). When CX717 was administered 10 min before the SD test session, the detrimental effects were eliminated($p<0.001$) and animals improved relative to their normal alert condition($p<0.001$). In the human study, 60 of 64 sessions were completed. On the MoW, the time to 30 secs. stage II sleep on P was 3.7 mins.; CX 717 1000mg showed a dose dependent increase to 7.3 minutes (p=.021). The overall effect of the three active doses vs.P was p=.08. During recovery PSG, SWS=103 minutes on P versus 81 minutes on CX 717 1000mg (p=.037);this was driven by reduced stage IV. A composite score across four cognitive tests revealed 300 ($p<.05$) and 1000 mg ($p<.01$)were superior to P. Both 100 mg (p=.06)&300 mg (p=.03) were associated with greater word retention. No serious adverse events were encountered.
Discussion: In these studies we demonstrated that pharmacological manipulation of AMPA receptor function improved cognition. The

ampakine CX717 dose-dependently enhanced performance in normal alert monkeys and reversed the task-specific impairments of SD.In turn, a SD study was conducted in human volunteers. As with the primate, low levels of performance induced by SD were improved by CX717 by the promotion of central arousal without autonomic activation. This is the first demonstrated pharmacocognitive effect of an AMPAkine & was facilitated by a primate DMS model.

18. Cholinergic Receptor Systems in Cocaine-Addicted Subjects: Alterations in Regional Cerebral Blood Flow

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Background: Reinforcement behaviors depend upon a balance between nucleus accumbens dopamine and acetylcholine (ACh), and reinforcement learning involves both muscarinic and nicotinic cholinergic receptors in the storage of drug-related information. Disruptions in cholinergic discharge and receptor systems may therefore play a key role in the addictive process. Recent preclinical studies demonstrate that the administration of cocaine itself induces marked changes in the cholinergic system, and drugs acting upon cholinergic receptors alter cocaine self-administration. In addition, our previous studies have revealed that the rCBF response to procaine, a limbic-stimulant with relatively high affinity to the cholinergic receptors, was different in cocaine-addicted subjects compared to controls. This study was designed to further assess putative changes in cholinergic receptors systems in cocaine-dependent subjects.
Methods: Cocaine-only addicted male subjects (25 to 45 y/o) were studied at two to six-weeks abstinence. On three separate study days, subjects were administered (1) the cholinergic muscarinic/nicotinic ACh agonist physostigmine i.v. over sixty minutes, (2) the muscarinic ACh agonist scopolamine i.v. over one minute, or (3) saline. The radioligand Tc-99m HMPAO was administered 50 (physostigmine) or 60 (scopolamine) min after drug infusion. Single photon emission computed tomography (SPECT) was used to compare the rCBF response to procaine and saline. SPM99 was used to assess differences within groups ($p<0.01$).
Results: Physostigmine Infusion: Control subjects (n=8) demonstrated an rCBF increase in left orbital frontal cortex (OFC), left thalamic, and right parahippocampal rCBF and a decrease in the rostral anterior cingulate and left DLPFC. Cocaine-dependent subjects (n=10) showed an increased rCBF in the left OFC (shared with the control group) as well as an increase in the right OFC and hypothalamus. Scopolamine Infusion: Controls showed an increased rCBF response in the right middle/posterior insula and decreased rCBF in the rostral anterior cingulate (similar to that observed following physostigmine), left caudate, and right thalamus. Changes in cocaine-dependent subjects (n=9) were limited to an increase in rCBF in the right DLFPC and a decrease in the right and medial OFC and right anterior (non-amygdalar) temporal cortex. Physostigmine vs. Scopolamine Infusion: Regions demonstrating an increase in rCBF following physostigmine relative to scopolamine infusion may reveal the effect of nicotinic excitatory neurons, whereas an rCBF increase following scopolamine relative to physostigmine may reflect activity of nicotinic inhibitory neurons. rCBF increases following physostigmine relative to scopolamine were observed in the thalamus bilaterally in cocaine-addicted subjects but in the left thalamus only in controls. Scopolamine relative to physostigmine demonstrated specific changes in the right amygdalar and DLPFC region in the cocaine-addicted subjects only.
Discussion: These findings suggest a regionally specific disruption of muscarinic and nicotinic receptor functioning in abstinent cocaine addicts. As these receptor systems are critical modulators of tonic and stimulated dopaminergic release, the identification of highly localized and functionally relevant regions of receptor dysregulation may provide specific targets for cholinergic pharmacologic treatments. These findings further imply that hypotheses suggesting ubiquitous, cortical-wide increases or decreases in receptor dysregulation may be overly simplistic. This work was funded by NIDA R01DA011434.

19. C-fos Activation Correlates with Cocaine Preference in Adolescent Rats

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Background: Abuse of stimulants often emerges during adolescence. Before this period, stimulants are reported to produce dysphoria in children, which transitions to euphoria with continued maturation. Part of this transition may be related to an increased awareness of the environment that occurs during the teenage years. While it is well established that prefrontal cortical regions play an important role in cue-induced drug-seeking behaviors in adulthood, it is unknown how strong this relationship is in younger ages. To determine whether an association exists between cortical activity and behavioral drug preference, we used c-fos immunoreactivity and place conditioning in juvenile, adolescent, and adult rats.
Methods: After establishing that no baseline preferences exist for either side of the conditioning chamber on day 1, juvenile (25 days), adolescent (40 days) and young adult (60 days) Sprague-Dawley male rats were placed in environments paired with either saline or 10 mg/kg cocaine for 60 min for two successive days. On the fourth day, they were tested in a drug-free state. Once preference or aversion to the cocaine-associated side was established for the first 30 min, they remained in that chamber for an additional 30 min. Immediately afterward, subjects were perfused, and the brains processed for c-fos immunoreactivity according to our standard laboratory procedures.
Results: Little activation was observed in the accumbens in the absence of drug. In contrast, c-fos expression was abundant in prefrontal regions of the cingulate gyrus (Cg1 and Cg2/3) and the infralimbic cortex in animals that exhibited a place preference to cocaine. Consistent with clinical observations, juvenile animals demonstrated a significant behavioral aversion to the cocaine-conditioned chamber and c-fos immunoreactivity did not correlate with cortical regions (Cg2/3 and infralimbic: $r<0.3$). By adolescence, a strong correlation ($r=.84$) was observed between c-fos immunoreactivity in Cg1 and level of drug preference or aversion, with higher c-fos expression predictive of place preference. Correlations between behavior and c-fos in Cg2/3 and infralimbic were not as strong.
Discussion: These data support our previous neurochemical findings that the prefrontal cortex assumes its adult-like function during adolescence, perhaps as a result of changes in D1 receptor expression and function. The preferential activation of the Cg1 region may be especially important for increased vulnerability to drug abuse that emerges during adolescence.

20. A Primer on Multivariate Methods for Pharmacoepidemiology: Cocaine Dependence Illustration

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Background: In truly multivariate statistical approaches for pharmacoepidemiology, investigators study one or more vectors of interdependent response variables or outcomes on the 'y' side of multiple regression equations, using subscripts to denote each vector's element. One analysis option is first to summarize the interdependent responses in a summary scale or index, or to summarize by using latent structure methods, and then to analyze inter-individual or inter-group variation in summary responses. In contrast to this 'first summarize, then analyze' approach, a complementary option is first to analyze individual interdependent responses, and then to summarize inter-individual or inter-group variation in the responses. The aim of this presentation is to clarify and illustrate the complementarity of

the 'first summarize, then analyze' approach and the 'first analyze, then summarize' approach, using data of a binary character often encountered in pharmacoepidemiological research — when responses to a drug compound are encoded as 'absent' or 'present' for each clinical feature under study.
Methods: Data are from national community sample surveys with standardized assessments of drug involvement and drug dependence, completed from 1995 through 2002. For example, between 1995-98, national sample surveys identified 927 recent-onset cocaine users; in 2001-2, n=1081 recent-onset cocaine users. Here, statistical approaches involve two options described above, including a generalized linear model with generalized estimating equations (GLM/GEE), as well as DSM summary indices and latent class methods. For illustration, we focus upon possible excess risk of dependence among crack-smoking cocaine users as an example of suspected inter-group variation in risk of a hazardous outcome.
Results: In application of the DSM summary index approach, among recent-onset users of cocaine, roughly 5%-6% had developed a DSM cocaine dependence syndrome soon after onset of cocaine use; among crack-smoking users versus cocaine HCl powder users, estimated risk was 3-4 fold greater ($p<0.05$). Under the latent class approach, roughly 6% had become cocaine dependent; dependence occurred 2-4 times more often among crack-smoking cocaine users ($p<0.05$). GLM/GEE approaches indicated 1.6-2.0 fold excess risk of cocaine dependence clinical features among crack-smokers ($p<0.05$), relative to occurrence of these same clinical features of cocaine dependence among those who had used only cocaine HCl powder. All relative risk estimates were robust when alternative suspected causal determinants of excess risk were held constant via multiple regression (e.g., with covariate adjustment for use of other drugs).
Discussion: Pharmacoepidemiological studies often involve multivariate responses of a binary (present/absent) form. There now are readily implemented and appropriate multivariate response models for analysis and summary of these multiple interdependent responses. By using the 'summarize, then analyze' option together with the complementary 'analyze, then summarize' option, pharmacoepidemiologists can shed new light on patterns of efficacious and hazardous responses to drug compounds.

21. Extinction-Induced Up-Regulation in AMPA Receptors Attenuates Sensitization to Cocaine and D2 Receptor Stimulation

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Sponsor: David Self

Background: Chronic cocaine use reduces excitatory AMPA glutamatergic input to medium spiny neurons in the nucleus accumbens (NAc) and induces long-term synaptic depression (LTD). These changes correlate with profound sensitization in dopamine D2 receptor-mediated responses that are involved in relapse to cocaine-seeking behavior. In contrast, we found that extinction training following chronic cocaine self-administration increases the GluR1 and GluR2 subunits of AMPA glutamate receptors in the NAc. This study investigated the potential interaction between cocaine-induced LTD and extinction-induced up-regulation in NAc AMPA receptors in the regulation of addictive behavior.
Methods: We utilized viral mediated over-expression of wild type GluR1 and GluR2, and a dominant-negative pore-dead GluR1 mutant to increase and decrease NAc AMPA receptor function, respectively. The effects of GluR over-expression in NAc neurons on cocaine, D1 (SKF 81297) and D2 (quinpirole) receptor responses were assessed in tests for both locomotor sensitization and reinstatement of cocaine-seeking behavior. In a separate experiment, extinction training was used to up-regulate endogenous GluR1 and GluR2 subunits in withdrawal from chronic cocaine self-administration to study potential effects on locomotor sensitization.
Results: Over-expression of GluR1 and GluR2 in NAc neurons facilitated D1 (SKF 81297), and attenuated D2 (quinpirole) receptor-stimulated locomotor responses. In addition, over-expression of both GluR1 and GluR2 blocked the development of cocaine and D2 receptor sensitization with repeated treatments. Similarly, extinction-induced up-regulation of endogenous GluR1 and GluR2 in the NAc attenuated cocaine and D2 receptor sensitization following chronic cocaine self-administration. In contrast, artificially mimicking cocaine-induced deficits in AMPA-mediated glutamatergic input by over-expressing dominant-negative pore-dead mutant GluR1 facilitated cocaine and D2 receptor sensitization. These locomotor results were paralleled in tests for reinstatement of cocaine-seeking behavior, where over-expression of GluR1 attenuated cocaine seeking induced by either cocaine priming- or the D2 receptor agonist quinpirole, but over-expression of dominant-negative GluR1 facilitated quinpirole-induced relapse to cocaine-seeking behavior.
Discussion: Taken together, these findings suggest that cocaine-induced deficits in excitatory AMPA receptor-mediated neurotransmission in the NAc facilitates sensitization in D2 receptors that trigger relapse to cocaine seeking. Conversely, restoration of these deficits by either viral-mediated over-expression or extinction training counteracts cocaine addiction by reducing sensitization in D2-mediated responses. These results also suggest that extinction training may have therapeutic efficacy in reversing the harmful neurobiological and behavioral changes associated with cocaine addiction.

22. Comparative Molecular Field Analyses of Amphetamine, Cocaine, and Antidepressant Recognition at Serotonin Transporter Mutants Reveals Contributions of Transmembrane Helix III to Ligand Binding

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Background: The serotonin transporter (SERT) is a member of the 12 transmembrane domain, Na^+/Cl^--dependent neurotransmitter transporter gene family. SERT functions to clear extracellular serotonin (5-HT) from the synapse, thus effectively terminating neurotransmitter activation of pre- and post-synaptic 5-HT receptors. SERT is a primary target of many drugs including cocaine, amphetamines, and antidepressants including the tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs).
Methods: Previously, we determined that several residues in the third transmembrane helix (TMH) of human SERT, including I167, A169, F170, and I172, influenced SSRI and cocaine analog potency. Recently, we have also identified that these same residues similarly affect drug potency for the TCAs (amitriptyline, clomipramine) and the amphetamines. Mutation of an additional residue S174 has also demonstrated a global decrease in amphetamine potency. Comparative molecular field analyses (CoMFA) utilizing selectivity fields were performed to compare the potencies of the drugs within the antidepressant, cocaine analog, and amphetamine drug classes to determine not only the steric and electrostatic determinants of the ligand interactions at these key residues, but also to gain a better understanding of the tertiary structural requirements for SERT ligand potency. CoMFA selectivity fields are useful in determining the molecular basis for differences in biological activities between a single point-mutant and the wild-type transporter and allow designed CoMFA models to distinguish between direct effects on ligand binding versus more global mutation-induced conformational effects on the binding pocket.
Results: Our results for the amphetamine CoMFA selectivity fields indicate changes in amphetamine potencies at the mutants I167L, A169D, I172M, and S174M are most likely due to conformational changes of the ligand binding pocket as no single CoMFA field predominated at each mutant. In contrast, for the F170I mutant, a very large positive electrostatic field surrounded the aromatic ring of the

amphetamine molecule indicating a direct need for increasing the positive character of the ring to compensate for the loss of the hydrophobic character resulting from the mutation. Likewise, our CoMFA model of the cocaine analogs indicated the changes in drug potencies at the mutants I167L, A169D, and S174M were due to global conformational changes in the tertiary structure of SERT. Once again, the F170I mutant displayed a very large steric bulk field surrounding the molecule indicating a loss of tolerance for bulky substituents at the mutant. Such a CoMFA selectivity field suggests that the mutation has severely restricted the space within the binding pocket. Additionally, a prominent negative electrostatic field was present in the CoMFA analysis of the I172M mutant that wrapped around the ester side chain and phenyl ring, but included an opening around the bridgehead nitrogen. Therefore, addition of negative charge or hydrogen bond acceptors on the molecule would increase cocaine analog potency at the I172M mutant by interactions with neighboring SERT residues.
Discussion: Specific conclusions for the antidepressant interactions could not be reached due to the poor alignment of the TCAs and the SSRIs. In summary, our results suggest major contributions of SERT ligand binding reside in TMH III. (Supported by grant MH60221).

23. Effects of Standard Dopaminergics and Comparison of D3 and D2 Receptor Ligands on Choice Between Concurrently Available Cocaine and Food in Rats

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Choice schedules of drug self-administration may be an especially useful approach for evaluating candidate treatments for drug abuse. First, allocation of behavior to drug taking at the expense of non-drug related behaviors is relevant for DSM-IV criteria for substance abuse. Second, the procedure described here was amenable to systematic comparison of acute and chronic administration, the latter being especially important for predicting the effects of a candidate medication in the clinic. Third, concurrent choice schedules measure response selection in addition to overall response rate, and thus may provide a measure of medication effects on cocaine's reinforcing efficacy independent of non-specific effects (e.g., general rate suppression). The goals of this study were two-fold: 1) to evaluate the effects of acute and chronic treatment with "standard" dopaminergic compounds that have been tested in clinical populations; and 2) to evaluate effects of novel dopaminergic agonists and antagonists with varying selectivities for the dopamine D3 receptor. First, acute and chronic treatment with the agonist d-amphetamine and antagonist SCH 39166 were evaluated. Second, acute treatment with agonists (PD 128,907, R-NPA) and antagonists (PG 01037, L-741,626) that differ in selectivity for dopamine D3 vs. D2 receptors was evaluated.
Methods: During sessions comprised of five 20-min components, Sprague-Dawley rats chose between i.v. cocaine (0, 0.032, 0.1, 0.32, 1.0 mg/kg) and liquid food (0.075 ml of 56% Ensure protein drink), and each component began with a single, noncontingent delivery of the two reinforcers.
Results: Under baseline conditions, rats chose primarily food when low cocaine doses (0, 0.032, 0.1 mg/kg) were available, and primarily cocaine when higher cocaine doses (0.32, 1.0 mg/kg) were available. Acute pretreatment with d-amphetamine (0.32-1.8 mg/kg) produced a dose-dependent leftward and upward shift in the cocaine choice dose-effect function, whereas chronic infusion of d-amphetamine for 7 days produced a dose-dependent rightward shift. Acute pretreatment with SCH 39166 (0.03-0.56 mg/kg), on the other hand, produced a small rightward shift in the cocaine choice dose-effect function, and effects of chronic infusion of SCH 39166 are currently being evaluated. Regarding D3 and D2 ligands, acute pretreatment with the D3-preferring agonist PD 128,907 (0.3-5.6 mg/kg) and the non-selective D2/D3 agonist R-NPA (0.01-0.3 mg/kg) produced dose-dependent leftward and upward shifts in the cocaine choice dose-effect function, a profile similar to that of d-amphetamine. The D3-selective antagonist PG 01037 (1.0-32 mg/kg) produced little or no alteration in cocaine choice up to doses that produced marked decreases in response rates. In contrast, the D2-preferring antagonist L-741,626 (0.3-3.2 mg/kg) produced a small, dose-dependent rightward shift, a profile similar to that of SCH 39166.
Discussion: In summary, the data with d-amphetamine are concordant with effects of chronic amphetamine on cocaine choice in rhesus monkeys and with clinical studies of amphetamine maintenance in human cocaine users, suggesting that this choice assay in rats yields data that may be predictive of clinical treatment efficacy. Secondly, that a D3-selective antagonist had no effect whereas a D2-preferring antagonist attenuated cocaine choice supports a role for the D2 receptor in this assay. Ongoing studies with chronic administration of selective D3 ligands are necessary to fully explore the extent to which the D3 receptor may or may not be a useful target for medications for cocaine abuse and dependence. NIDA DA07252, DA14644 and the Zaffaroni Foundation.

24. Behavioral And Neurochemical Effects Of Chronic Exposure To D2 Agonist Treatment In Monkeys

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Background: Considerable evidence has accumulated to suggest a prominent role for dopamine D2 mechanisms in the behavioral effects of psychomotor stimulant drugs such as the monoamine transport blockers methamphetamine (MA) and cocaine (COC). The present experiments were conducted to further evaluate the involvement of D2 mechanisms in psychomotor stimulant action by examining the development of tolerance and cross-tolerance to behavioral effects of different dopaminergic drugs in monkeys exposed to a chronic regimen of treatment with the D2-family agonist (+)-PHNO.
Methods: The effects of a range of acutely administered i.m. doses of (+)-PHNO (0.0001-0.03 mg/kg), the D2-family agonist quinelorane (0.003-0.3 mg/kg), the D1-family agonist SKF 82958 (SKF; 0.03-1.0 mg/kg), and the psychomotor stimulants methamphetamine (MA; 0.01-0.3 mg/kg) and cocaine (COC; 0.03-1.0 mg/kg) were studied in three groups of monkeys prior to and during 1-2 months of chronic treatment with 0.003 mg/kg/hour of (+)-PHNO administered via osmotic minipumps. Effects of drugs were evaluated on overt behavior (self-scratching) associated with D2-family receptor activation in one group of monkeys, and on operant behavior (leverpressing maintained under a fixed-ratio schedule of stimulus-termination) in a second group of monkeys. A third group of monkeys was trained to distinguish injections of 0.32 mg/kg of MA from vehicle using drug discrimination procedures. Following re-determination of the effects of drugs during the chronic regimen in the third group (n=4), ex vivo autoradiographic analyses were conducted to evaluate changes in D2 receptor density that may have occurred as a result of continuous exposure to (+)-PHNO.
Results: Results indicate that tolerance to the effects of (+)-PHNO , indicated by at least a 10-30 fold rightward shift in the dose-response function occurred on all behavioral measures, including self-scratching, response rate disruption, and MA-like discriminative-stimulus effects. This tolerance was associated with significant reductions in dopamine D2 receptor levels in selected brain regions, including caudate (by 25%, $p<0.01$), putamen (by 30%, $p<0.005$) and accumbens (by 41%, $p<0.003$). In contrast to the tolerance observed with (+)-PHNO and cross-tolerance noted for quinelorane, no tolerance was evident in behavioral effects produced by the psychomotor stimulant drugs methamphetamine and cocaine, including response rate disruption and MA-like discriminative effects.
Discussion: These data indicate that, whereas D2 mechanisms are considered to play an important role in the behavioral effects of psy-

chomotor stimulant drugs, non-D2 mechanisms must be intimately involved in the behavioral actions of indirectly-acting dopaminergic psychomotor stimulant drugs including methamphetamine and cocaine. Such mechanisms include those that mediate MA discrimination and likely are also involved in the abuse-related effects of these psychomotor stimulant drugs. (These studies were supported by NIH/NIDA DA03774 and DA10566, and were carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.)

25. Neurotensin System Genes and Susceptibility to Cocaine-induced Psychosis

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Background: Neurotensin (NT) is a 13 amino acid neuropeptide with close anatomical and functional associations with the mesolimbic dopamine system and has been implicated in the mechanism of action of antipsychotic drugs and the pathophysiology of schizophrenia and drug abuse. Through its modulatory effects on mesolimbic dopamine pathways, the NT system is in a prime position to influence the development of symptoms due to over-stimulation of that dopamine system, such as cocaine induced psychosis (CIP). Over 60% of chronic cocaine abusers will suffer from CIP and twin and association studies suggest a genetic susceptibility for this symptom.
Methods: To determine a possible association of polymorphisms within the NT system and CIP, we genotyped single nucleotide polymorphisms (SNPs) in the NT gene (NTS; 12q21) and the genes of three of its receptors (NTSR1, 20q13; NTSR2, 2p25 and SORT1, 1p13) in 238 cocaine abusers. In these subjects, CIP was assessed using the Scale of Assessment for Positive Symptoms in CIP (SAPS-CIP).
Results: We first established dense marker maps for these genes in European (EA) and African Americans (AA) in order to develop population specific tagging SNPs. Average marker density per gene ranged from 2-5 kb and for each gene linkage disequilibrium extended over a much shorter distance in AA than EA. Association of these SNPs with CIP was assessed separately for EA ($N = 90$) and AA ($N = 127$). Initial results show significant associations ($p<0.05$) of polymorphisms within NTSR2 and SORT1 with the development of CIP.
Discussion: These data suggest a possible modulatory role of the NT system in CIP.

26. Relationships Between State Policies and the Availability of Services for HIV/AIDS, Hepatitis C Viral Infection, and Sexually Transmitted Infections in Substance Abuse Treatment Programs

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Background: This report examines the associations between HIV/AIDS, hepatitis C (HCV), and sexually transmitted infection (STI)-related services provided by substance abuse treatment programs in the National Institute on Drug Abuse Clinical Trials Network (NIDA CTN), and the states within which they are located.
Methods: Substance abuse treatment programs from nearly 100 different agencies within the NIDA CTN participated in this study. Administrators of state substance abuse and/or health departments from 48 states and the District of Columbia also participated. Data for this report was derived from two surveys: one for substance abuse treatment program administrators, and one for state health and substance abuse department administrators. The surveys included cross-sectional, descriptive survey of infection-related services: patient risk assessment, biological testing, education, counseling, medical exams, treatment and monitoring; treatment program structure, setting, and staffing; and infection prevalence of the patients. The analysis included descriptive statistics for survey variables; principal component, cluster or factor analysis to group and reduce the number of variables, and structural equation models to test for associations.
Results: 265 of 313 (86%) substance abuse treatment program administrators responded, from 95 agencies in the NIDA CTN, covering 26 states & DC. Services such as risk assessment and patient education were offered (or referral provided) for the three infections groups in more than 75% of the substance abuse treatment programs, whereas infection-related pharmacotherapies and clinical monitoring were offered (or referral provided) in less than half of the programs. HIV/AIDS, HCV and STI risk assessments are mandated by 55%, 28%, and 33%, respectively, of the states responding. In contrast, 89%, 77%, and 77% of substance abuse treatment programs offer risk assessments targeting HIV/AIDS, hepatitis C, and STI, respectively.
Discussion: There is substantial variation in the % of programs offering the various services for a particular infection group, however; there is consistency in the % of programs offering a particular service for all three infection groups. This information suggests that the presence of state polices can be used to encourage "best practices" in treating these epidemic infections.

27. N-3 Polyunsaturated Fatty Acids Decrease Feelings Of Anger In A Population Of Substance Abusers

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Sponsor: Alec Roy

Background: It has been suggested that low levels of some polyunsaturated fatty acids (PUFAs) play a role in the pathophysiology of depressive, suicidal and aggressive behaviors, including homicides. This can be understood in light of the fact that PUFAs are structural components of neuronal membranes phospholipids and influence neuronal function. The evidence linking low levels of PUFAs and aggression is however still scant. Because aggressive behaviors are intensively bound up with drug use, we studied the effects of the administration of PUFAs of the n-3 series in a group of substance abusers.
Methods: Thirteen patients were given on a daily basis capsules containing 3 grams of n-3 PUFAs (2,250 mg of eicosapentaenoic acid (EPA, 20:5n-3), 500 mg of docosahexaenoic acid (DHA, 22:6n-3) and 250 mg of other n-3 PUFAs). Eleven patients received similarly looking placebo capsules containing vegetable oils. The treatment duration was 3 months. Capsules were given in a double-blind manner. A modified version of the Profiles of Mood States (POMS) questionnaire was administered at baseline and every month thereafter for a period of 3 months. Six patients in the PUFA group and 8 patients in the placebo group were followed up for an additional 3 months after treatment discontinuation and were administered the same questionnaire every month. At baseline, a life long history of aggressive behavior and a history of problems with the law were obtained.
Results: The 13 patients who received n-3 PUFAs and were followed for 3 months showed a progressive decline in the POMS anger subscale scores. This was not the case for the 11 patients who received placebos. A comparison of the 2 groups by repeated measures ANCOVAs (with baseline value as covariate) revealed a significant difference ($p<.025$). The 6 patients in the PUFA group who were followed for 6 months showed a progressive increase in anger scores from the 4th to the 6th month, but their scores did not return to baseline levels. No trend was observed in the anger scores fluctuations recorded from the 4th to the 6th month in the 8 patients who had received placebo capsules. A comparison of the 2 patient groups followed for 6 months was significant at a trend level. Four of the 13 patients in the PUFA group and 4 of the 11 patients in the placebo group had a history of assaultive behavior and 7 patients in each of these groups had

been jailed for offences ranging from drug possession to theft, DWI, weapon possession and assaults.
Discussion: This study, which needs to be replicated in larger samples, showed that the daily administration of 3 grams of n-3 PUFAs for a period of 3 months significantly decreased feelings of anger in a population of substance abusers by comparison with the administration of a placebo. This decrease was followed by an increase in anger scores in a subgroup of patients who were followed for an additional 3 months. These data give support to epidemiological studies showing a decrease in homicides in countries where the consumption of foods rich in n-3 PUFAs such as fish is high. Angry feelings can lead to aggressive behaviors. Supplements of n-3 PUFAS, that are both inexpensive and well tolerated, might be considered as treatment adjuncts in patients displaying these behaviors.

28. Reduced Cocaine Self-Administration in Muscarinic M5 Acetylcholine Receptor-Deficient Mice
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Background: The reinforcing effects of cocaine have been related to increased extracellular concentrations of dopamine in the ventral striatum. Several studies suggest that M5 muscarinic receptors facilitate striatal dopamine release. We tested the hypothesis that the reinforcing effects of cocaine are decreased in M5 receptor-deficient mice using chronic intravenous cocaine self-administration in extensively backcrossed mice. We also assessed whether operant performance generally, rather than cocaine self-administration specifically, was altered in the mutant mice.
Methods: To this end we evaluated both food-maintained operant behavior and cocaine self-administration under a fixed ratio (FR) 1 and a progressive ratio (PR) schedule of reinforcement. We also evaluated acquisition of self-administration in experimentally naïve mice using several doses of cocaine.
Results: M5 receptor deletion decreased self-administration of low to moderate doses of cocaine under a PR schedule of reinforcement and diminished acquisition of self-administration of a low dose in experimentally naïve mice. We found no differences between genotypes in food-maintained behavior.
Discussion: The present results confirm and extend our previous findings, using backcrossed mice and covering various experimental conditions. Our results indicate that M5 receptor deletion diminished the reinforcing effects of low doses of cocaine, and identified specific conditions under which this may be observed. The present work was supported by grants from NIDA/NIH (DA12142, DA14644), the Zaffaroni Foundation and the Lundbeck Foundation.

29. Decreased CNS Serotonin Turnover, Early-Life Stress, and Initial Response to Ethanol are Predictors of Ethanol Consumption in Adolescent Rhesus Macaques
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Sponsor: Barbara Mason

Background: Response to alcohol has been demonstrated to be a strong predictor of alcoholism in human subjects.
Methods: To examine the relationship between serotonin turnover, response to the motor-impairing effects of ethanol, and ethanol consumption, cerebrospinal fluid (CSF) concentrations of 5-hydroxyindoleacetic acid (5-HIAA) were determined for peer- and mother-reared, ethanol-naive rhesus macaques (N=27; 3 years of age at the start of study). One month after CSF samples were obtained, animals were administered an intravenous dose of ethanol (males: 2.2 and females: 2.0 grams ethanol per kg body weight using a 16.8% ethanol solution, that produced homogeneous blood alcohol concentrations between individuals and the sexes) and then rated for degree of intoxication. The total volume of ethanol solution was infused over a 20-min period. Subjects then were trained to consume ethanol by first exposing them to an aspartame-sweetened vehicle, until they freely consumed the vehicle. Thereafter, sufficient ethanol was added to produce an 8.4% ethanol water-aspartame solution, and the subjects were allowed to drink the ethanol water-aspartame solution (1 hr/day; 5 days/week) across two consecutive weeks.
Results: CSF 5-HIAA concentrations were positively correlated with ratings for intoxication following the alcohol infusion (i.e., low CSF 5-HIAA predicted minimal intoxicating effects). Low CSF 5-HIAA and decreased level of response to alcohol were predictive of future voluntary alcohol consumption.
Discussion: Our findings suggest that low CNS serotonin turnover rate is related to a decreased response to alcohol and is a potential risk factor for increased alcohol consumption without the confound of prior exposure to ethanol. All research was carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.

30. Pain and Opioid Addiction: Complex Relationship
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Sponsor: James Anthony

Background: Prescription opioid abuse has markedly increased over the past decade, and a key concern is to understand the epidemiology of pain-that is the very persons who are likely to be prescribed opioids: How many people suffer from significant pain? What are the socio-demographic correlates of pain? How much is pain associated with mood disorders, anxiety disorders, personality disorders and substance use disorders? Is opioid misuse and opioid abuse and dependence associated with pain?
Methods: Data come from the National Epidemiological Study of Alcohol and Related Conditions (NESARC), a nationally representative study of the U.S. household population ages 18 and older (N = 43,093). Rates of pain were calculated for the population as a whole and according to sociodemographic subgroups as well as according to the presence/absence of substance misuse and multiple DSM-IV disorders: alcohol and specific drug disorders, personality disorders, mood disorders, and anxiety disorders. The measurement of pain was based on self-report of functional impairment during the past four weeks because of somatic pain. Multinomial regression was used to calculate odds ratios to estimate the strength of the association between pain and each of the possible risk factors, adjusting for the presence of the other conditions (i.e. all the co-morbidities). Standard errors were calculated using SUDAAN.
Results: Pain is a very common phenomenon: 34.8% of the adult U.S. population (71.2 million persons) report functional impairment in the past four weeks due to pain and 12.2% (25.0 million persons) report "quite a bit" or "extreme" levels of impairment. Pain is more commonly reported by women and older age groups. African Americans are more likely than whites to report pain; while Hispanic ethnicity is not associated with significant differences. Mood disorders, anxiety disorders, and personality disordersis are all associated with increasing reports of pain. Substance misuse and substance use disorders are inconsistently associated with reports of pain: controlling for comorbidity, only opioid misuse and opioid disorders are significantly related to pain. Alcohol and other illicit substance misuse and disorders are not consistently related to reports of pain.

Discussion: This is the first epidemiological study of the U.S. population to address the relationship of pain to psychiatric disorders, including substance use disorders. Findings indicate that most psychiatric disorders are associated with reports of pain. Opioid misuse and opioid abuse and dependence were also found to be associated with pain. Thus, pain does not protect from opioid disorders but can be seen as a possible risk factor. Limitations of the study include minimal measures of the pain phenomena, lack of assessment of chronic health conditions, and concerns about how the diagnosis of abuse and dependence apply to persons who may be prescribed opioids for long periods of time. Future work will need to disentangle the factors which mediate the relationship of pain and opioid use disorders. Is this due to opioid abusers being at risk for injury and suffering pain? This is unlikely because none of the other substances were similarly associated with pain and yet these are commonly associated with physical injury. Perhaps persons in pain are at higher risk for being prescribed opioids and some portion of these individuals will develop the typical problems of opioid misuse and addiction. This does not rule out a potential dampening of the addictive potential of opioids in the presence of pain but indicates that pain is not completely protective. For clinicians and clinical researchers, a goal will be to balance the obvious benefits of opioids for controlling pain with the risk of abuse or dependence in vulnerable individuals.

31. Attenuation of Ethanol and Sucrose-Seeking and Intake Following Chronic Acamprosate or Naltrexone Treatment

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Sponsor: Past Travel Awardee, Memorial, 2003

Background: Two pharmacotherapies are currently approved for use in treating alcohol "craving". Naltrexone is an opiate-receptor antagonist that has been shown to be useful clinically for attenuating alcohol craving and postponing relapse and in animal models to decrease alcohol intake. Acamprosate has been used to treat relapse in detoxified alcoholics and it has been shown to decrease alcohol, but not food or water self-administration in rats. The present experiments utilized a "reinforcer-blocking" approach to better understand the mechanism of action and efficacy of these treatments for ethanol-seeking and drinking using nondependent rats as subjects.
Methods: Drugs were administered daily over 3-week periods (5 day/week; 3 doses each with 2 weeks of saline between doses) prior to operant sessions where rats had to perform a low response requirement (2 lever-presses) to gain access to ethanol for 20 min. Therefore, rats were able to consume ethanol during drug treatment (sucrose control animals underwent same treatment). Then, on Mondays following 14 drug/reinforcer sessions and approximately 72hr without drug or reinforcer solution exposure, a single extinction session was conducted (20 min of access to the lever with no reinforcer presented). This measure allowed for the determination of appetitive response strength, or the tendency to seek ethanol, following the chronic drug/reinforcer pairings.
Results: Naltrexone decreased both ethanol and sucrose intake at all doses (-30min; 0.1, 0.3, 1.0 mg/kg) relative to saline. In both groups over the 3 weeks of naltrexone treatment, there was no evidence of tolerance (i.e., the non-selective decrease in intake of both sucrose and ethanol was stable from the first to last day of naltrexone treatment). When the seeking response was assessed in the extinction sessions, naltrexone had no effect in the sucrose group while in the ethanol group, naltrexone significantly decreased seeking at all doses (lever presses - saline: 82 +/- 10; 0.1Nal: 57 +/- 8; 0.3Nal: 56 +/- 13; 1.0Nal: 42 +/- 9). This change in extinction responding indicates a change in incentive motivation resulting from the reinforcer devaluation caused by drug/reinforcer pairings, which was selective for the ethanol group. The acamprosate experiment is underway, and preliminary data indicate that the low dose (-2 and -21hr; 50 mg/kg) had no effect on ethanol-seeking or intake with similar levels of baseline responding as seen in the naltrexone study. This dose also had no effect on sucrose-seeking, with a tendency to decrease sucrose intake. Doses of 100 and 200 mg/kg will be examined as well.
Discussion: This paradigm may model the early stages of "problem drinking" in humans, where individuals are engaging in heavy consumption but are not yet dependent and possibly seeking treatment, which could provide information about the best intervention for this level of alcohol abuse and relapse prevention. *All procedures in accordance with NIH Guide; Supported by NIAAA AA013860*

32. Insulin And Amphetamine: What's Up With Dat?

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Background: The dopamine (DA) transporter (DAT) is a primary site of action of drugs of abuse such as amphetamine and is critical in regulating DA neurotransmission by high affinity transport of DA into the terminal. Understanding how the DAT is regulated is, therefore, of fundamental importance to studies of amphetamine abuse. In this regard, there is converging evidence that insulin can exert profound regulatory control of DAT activity. It is known for example that both food restriction as well as experimentally-induced diabetes have marked effects on behavioral responses to amphetamine. Recently we reported that rates of DA uptake are decreased in hypoinsulinemic rats and that insulin increased DA uptake in cells stably transfected with the DAT. Based on these findings we hypothesized that insulin regulates expression of the DAT in the plasma membrane.
Methods: Rats were rendered either hypoinsulinemic by a single injection of streptozotocin (STZ) or hyperinsulinemic by subcutaneous implant of an insulin containing pellet (Linplant). In vivo high-speed chronoamperometry was used to measure clearance of exogenously applied DA or amphetamine-evoked endogenous DA release from striatum of anesthetized rats.
Results: Amphetamine (50 pmol) elicited 5-fold less DA release in STZ-treated rats compared to control rats (1.0 ± 0.3 vs 4.9 ± 0.5 μM respectively). Moreover, clearance of amphetamine-evoked DA was also significantly reduced in STZ-treated rats (2.5 ± 0.8 nM/sec, n=5) compared to saline-treated controls (16.3 ± 4.9 nM/sec, n=6). Given that STZ-treatment does not reduce tissue DA levels or change total DAT expression, these results are consistent with the idea that plasma membrane expression of the DAT is reduced in conditions of hypoinsulinemia. In contrast, the rate of DA clearance was increased two-fold in hyperinsulimic rats compared to control rats. A remarkable finding from our earlier studies was that the reduced rate of DA clearance in hypoinsulinemic rats could be restored to those of control rats by a modest dosing regime of amphetamine (1.78 mg/kg, ip, every other day for 8 days). Because the D2 autoreceptor can regulate DAT function and can activate the insulin signaling cascade [e.g. Akt through mitogen-activated protein kinase (MEK)] we focused our initial studies on a potential role for this receptor in mediating the ability of amphetamine to restore DA clearance rates in hypoinsulimic rats. Rats received the same amphetamine dosing regime as before; however, subgroups of rats received either the D2/D3 antagonist, raclopride, or saline 30 minutes earlier. Raclopride completely blocked the ability of amphetamine to restore DA clearance rates in hypoinsulinemic rats. Interestingly, raclopride by itself significantly reduced the rate of DA clearance. Given that no drug was present at the time DA clearance was measured, these results suggest that relatively short term blockade of the D2/3 receptor leads to long lasting changes in DAT function. Similarly, down-regulation of insulin signaling by removal of insulin (STZ-treatment) also appears to reduce DAT activity.
Discussion: Taken together these data suggest that DAT activity is tightly linked to insulin and/or insulin signaling pathways. Insulin and its signaling pathways may represent novel targets for developing

new treatments for drug abuse and importantly, may help illuminate the neural mechanisms underlying drug abuse and the high co-morbidity of eating disorders (where insulin levels can change dramatically). Support Contributed By: NIH grants R21 DA018992 (LCD), RO1 DA14684 (AG) and K05 DA17918 (CPF).

33. Stress, Alcohol, and Fluvoxamine: Composing the Score for a Dissonant ConSERT

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Background: The serotonin (5-HT) transporter (SERT; 5-HTT) provides the primary mechanism for terminating 5-HT neurotransmission and there is evidence that it is under regulatory control of hormones released in response to stress. Stress is linked to alcoholism and there is growing evidence that genetically driven SERT expression may be an important predisposing factor for stress and/or alcohol-related disorders. For example, humans and non-human primates with a low-functioning form of the SERT exhibit altered sensitivity to the intoxicating effects of ethanol and an increased propensity to consume ethanol. Moreover, recent studies indicate that the effects of SERT gene-deficiency may be further modified by exposure to stress. Here we examine the relationship between chronic stress, SERT function and sensitivity to ethanol in vivo mouse models.
Methods: Male C57BL/6J mice were stressed (daily 10 min forced swim for 14 days) or were unhandled. Twenty four hours following the final stress session, mice were either prepared for chronoamperometry to measure 5-HT clearance in CA3 region of hippocampus, or tested in behavioral assays. The loss of righting reflex was used as a measure of the sedative/hypnotic effect of ethanol. Parallel studies using SERT mutant mice (C57BL/6J background), were carried out in non-stressed, unhandled mice. SERT heterozygotes express 50% fewer SERTs than wild-type litter mates and SERT KOs lack the SERT. Quantitative autoradiography was used to assess total SERT expression in all mice.
Results: Stressed non-mutant C57BL/6J mice mirrored SERT KO mice in that 5-HT clearance was drastically reduced relative to control mice. Total SERT expression was not different between stressed and non-stressed mice. Thus, it appears that the reduced rate of 5-HT clearance in stressed mice may be attributed to either reduced plasma membrane expression and/or reduced intrinsic activity of the SERT. Relative to wild-type littermates, SERT KO mice displayed increased sensitivity to the sedative/hypnotic effects of ethanol, while the ability of ethanol to retard serotonin clearance was significantly potentiated in SERT heterozygote and KO mice. The effects of chronic swim stress on behavioral responses to ethanol in non-mutant C57BL/6J mice were similar to those of SERT KO; stress significantly increased sensitivity to the sedative/hypnotic effect of ethanol. Altered metabolism of ethanol could not account for these differences. By contrast, the effect of ethanol on 5-HT clearance was not different between stressed and non-stessed mice. Interestingly, the ability of the selective 5-HT uptake inhibitor, fluvoxamine, to inhibit 5-HT clearance was augmented in stressed mice, compared to non-stressed mice. This result parallels our previously published finding where SERT heterozygote mice also showed an exaggerated response to fluvoxamine. Fluvoxamine did not alter 5-HT clearance in SERT KO mice.
Discussion: These data suggest that reduced SERT function, as a consequence of either genetic or environmental manipulation leads to an enhanced response to the 5-HT uptake inhibiting effect of fluvoxamine and enhanced behavioral responses to ethanol. In contrast, adaptive changes that occur in response to a genetic reduction in SERT expression appear requisite for the enhanced ability of ethanol to inhibit 5-HT clearance. This latter finding is highly intriguing given growing evidence that a low-functioning variant in the promoter region of the human 5-HTT gene is positively associated with risk for various neuropsychiatric diseases, chiefly mood disorders and alcoholism. Support Contributed By: RO1-MH64489 & NARSAD (LCD) and NIAAA-IRP (JMB-R, AH).

34. An Association Study of Adenosine Receptor Gene Polymorphisms and Acute Responses to Caffeine in Light- or Non-Caffeine Users

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Background: Caffeine is the most widely used psychoactive substance in the world. Although it produces mild stimulant effects in most individuals, caffeine also produces anxiety or other adverse effects in some individuals. In a previous study we (Alsene, Deckert, Sand and de Wit, Neuropsychopharmacology 28, 1694-1702, 2003) found that adverse responses to caffeine (150 mg oral) were associated with polymorphisms in a gene encoding an adenosine receptor, where caffeine has its primary action. Healthy non-daily caffeine users with a polymorphism of the 1976C ⟩T genotype in the A_{2A} adenosine receptor gene experienced anxiety after a single low dose of caffeine (150 mg caffeine).
Methods: The present study was designed to replicate and extend these findings by examining responses to acute doses of caffeine (0, 50, 150 and 450 mg) in a new sample of 102 healthy volunteers who consumed less than 300 mg caffeine (i.e., less than 3 cups of coffee) per week. Doses were tested double blind, in randomized order, and subjective and behavioral measures were obtained for 3 hours after each drug administration.
Results: Caffeine dose-dependently increased subjective ratings of Feeling the drug effect, Arousal, Stimulation and Anxiety, and decreased ratings of Liking the drug effect. It increased systolic blood pressure but decreased heart rate. On behavioral tasks caffeine decreased Digit Span (memory), but increased the number of hits and decreased reaction time on a vigilance task. Responses to caffeine were examined in relation to several genotypes, consisting of polymorphisms in the adenosine A_1 (716T⟩G) and A_{2A} (1976T⟩C; 2592C⟩T; 263C⟩T; Intron; 9,5kb 5'-flank; 5,5kb 3'-flank) receptor genes. As in the previous study, subjects with the 1976TT genotype reported more anxiety after 150 mg caffeine. Preliminary analyses suggest there were no significant associations between genotypes and responses to caffeine at other doses, after correction for multiple testing. An extended haplotype analysis is pending.
Discussion: These findings replicate and extend our previous finding that polymorphisms in the adenosine receptor gene are related to subjective responses to acute caffeine administration. The study illustrates a powerful approach to investigating individual variations in responses to psychoactive drugs.

35. Dual Diagnosis and Drinking Behaviors in an Outpatient Treatment Seeking Sample of Adolescents with Alcohol Use Disorders

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Background: Co-occurring psychiatric disorders in adolescents with substance abuse is common. While many studies have explored the prevalence of psychiatric disorders in adolescent substance using samples, few have explored the relationship between comorbid psychiatric disorders and drinking behaviors in adolescents.
Methods: We examined 34 outpatient adolescents with alcohol use disorders for comorbid psychiatric disorders using the K-SADS. Their drinking behavior patterns were examined using the Time-Line follow-Back. The alcohol drinking parameters were (1) drinks per drinking day (DDD), (2) percent heavy drinking days (PHD), (3) percent heavy drinking days when drinking (PHDD), and (4) percent days abstinent (PDA).
Results: The diagnoses that afforded sufficient power to examine the effect of that diagnosis on drinking behavior were any mood or anxi-

ety disorders vs. neither; oppositional defiant disorder (ODD) vs. no ODD; and attention-deficit hyperactivity disorder (ADHD) vs. no ADHD. Results revealed no significant effect of either ODD or any mood anxiety disorder on drinking indices, both p values > .10; MONOVA revealed a significant effect of ADHD diagnosis, p=.04. Univariate analysis showed that for all four drinking indices, the group with ADHD had more severe alcohol use, all p values <.05.
Discussion: Our results suggest that adolescents with ADHD who meet diagnostic criteria for alcohol use disorders have greater drinking severity than those without ADHD.

36. A Double Blind Placebo Controlled Trial of Bupropion Added to Nicotine Patch and Cognitive Behavioral Therapy in Smokers with Current or Past Unipolar Depressive Disorder

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Sponsor: Maurizio Fava

Background: There is a strong relationship between depression and smoking[1,2]. People with depression are more likely than those in the general population to smoke. Smokers have more anxiety and depression than non-smokers, and smoking may reduce negative affect among those with depressive disorders[3]. Depressed smokers are less likely to successfully quit smoking, and smoking cessation may worsen depressive symptoms and increase risk for relapse to depression[4-7]. However most treatment trials exclude depressed smokers.
Methods: Our objective was to determine if bupropion added to nicotine patch (NRT) and cognitive behavioral therapy (CBT) is more effective than NRT and CBT alone for treatment of nicotine dependence in patients with unipolar depressive disorders (UDD). Adult smokers (n=199) (>0.5 ppd) were stratified according to current (n=90) or past (n=109) UDD, heavy nicotine dependence (n=106) and past nicotine dependence treatment failure (n=67) and randomly assigned to receive bupropion (n=97) or placebo (n=102) added to NRT and group CBT for 12 weeks. Dropouts were analyzed as smokers; 99 subjects completed the 12-week trial.
Results: Seventy-two percent (143/199) achieved 7-day point prevalence abstinence at least once during the trial. Ninety-seven percent of completers had >50% reduction in cigarettes smoked per day at the end of the trial. Thirty-four percent (67/199) had 7-day point prevalence abstinence at the end of treatment, and 15% (30/199) achieved continuous abstinence for the last 4 weeks of treatment, the primary outcome measure. Among those with current UDD, 32% were abstinent at end of treatment compared with 34% of those with past UDD. NRT usage was associated with abstinence: for each patch used, the OR of 7-day point prevalence abstinence at end of treatment was 1.06 (95% CI: 1.03 -1.08, p<0.001). Diagnosis of any anxiety disorder at baseline was associated with failure to attain abstinence at the end of treatment: 23% (18/79) of participants with an anxiety disorder achieved abstinence at the end of treatment vs 41% (49/120) of those without an anxiety disorder, Chi2= 6.9, p<0.01. Abstinence was associated with development of new episodes of major depressive disorder (MDD) in those who entered the trial with past but not current UDD; 22% (15/67) of participants who were abstinent at end of treatment developed MDD during the trial vs 10% (13/132) who were not abstinent, Chi2=5.8, p=0.02.
Discussion: In this trial of nicotine dependence treatment in depressed smokers, 33% attained abstinence at the end of treatment. NRT usage increased odds of abstinence. Diagnosis of any comorbid anxiety disorder had a strong effect to reduce abstinence rates. Abstinence was significantly associated with development of MDD during the trial. Analysis of medication effects, longitudinal analyses of the effects of abstinence on psychiatric symptoms and data from the 12-month follow up will be presented. Hughes, J. 1986. Am J Psychiatry. 143: 993-997. Glassman, A. H. 1993. Amer J Psychiatry. 150: 546-53. Lerman, C., et al. 1996. Addict Behav. 21: 9-19. Hall, S. M., et al. 1993. J Consult Clin Psychol. 61: 761-7. Glassman, A. H., et al. 2001. Lancet. 357: 1929-32. Haas, A. L., et al. 2004. J Consult Clin Psychol. 72: 563-70. Kahler, C. W., et al. 2002. J Abnorm Psychol. 111: 670-5.

37. Genomic Screen for Loci Associated with Marijuana, Stimulant and Drug Dependence in Mission Indians: Relation to Body Mass Index

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Background: Substance abuse is one of the most urgent health problems facing Native Americans, yet the underlying etiology of the disorder remains elusive. Complex disorders like marijuana and stimulant dependence may be influenced by a number of genes that may be specific to the etiology of those disorders or could overlap with other addictive/consumptive disorders. One general theory of drug addiction posits that the neurobiological mechanisms underlying the homeostatic regulation of appetitive drives and instincts to consume food and beverages becomes disregulated during the process of drug exposure. One theoretical assumption concerning Native people is that the long history of dependence on foraging and subsistence agriculture may have led to selective enrichment of traits that improve genetic fitness, so called thrifty or fat sparing genes. It has been suggested that this same selective pressure may have enriched for genetic variants that increase the risk for consumption of drugs.
Methods: This studys aims were to: 1) map susceptibility loci for DSM-III-R marijuana (MJ) and stimulant (STIM) dependence, and 2) to contrast the findings to loci mapped using more general consumptive/ addictive phenotypes e.g. the presence of: any drug dependence and/or regular tobacco use, and body mass index (BMI), in Mission Indian families. Each participant was assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). Genotypes were determined for a panel 791 microsatellite polymorphisms. Variance component analyses from SOLAR were used to generate LOD scores.
Results: Evidence for linkage was found on chromosome 6 for both the: any drug (LOD score= 2.5) and BMI (LOD score = 2.3) phenotypes. Bivariate analyses of the two phenotypes revealed a combined LOD score of 4.1 at that location. A locus on chromosome 14 was found for MJ dependence (LOD score =2.1) and one on chromosome 1 (LOD score = 1.5) was for STIM dependence. Additional loci on chromosomes 6, 11,13, 15, 16, 21, 22 were found for the: any drug phenotype, and on chromosomes 8, 16, 18 for BMI (LOD scores ranged between 1.0-2.7). Bivariate analysis of the BMI and any drug phenotypes produced a combined LOD score of 2.9 on chromosome 18.
Discussion: These data represent the first family-based genome-wide chromosome segregation analyses using MJ and STIM dependence phenotypes in any ethnic group. The results corroborated the possible importance of several chromosomal regions highlighted in prior linkage studies for substance abuse phenotypes and identify new regions of the genome for drug dependence. The fact that no overlaps were found in sites identified for MJ, STIM, and ALCOHOL suggest that these substances may have unique genetic factors associated with dependence on that substance. However, the fact that the more general phenotypes of ANY DRUG/REG TOB and BMI overlapped in several chromosomal locations also lends support for the hypothesis that some aspects of drug usage and food consumption may share common risk and/or protective factors in this Mission Indian population (supported by DA019333, AA10203.

38. The Effects of Exogenous Progesterone on Cocaine Self-Administration in Women

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Background: In a recent study we showed that exogenous progesterone attenuated the positive subjective effects of smoked cocaine in

females, but not in males. The purpose of the present study was to determine if exogenous progesterone would decrease actual cocaine self-administration in non-treatment seeking female cocaine smokers.
Methods: A cocaine self-administration dose-response curve was conducted during 1) a normal follicular phase, 2) a normal luteal phase and 3) a follicular phase made to resemble the luteal phase by the administration of oral micronized progesterone. The order of menstrual cycle phase was counterbalanced across women and the order of cocaine doses was randomized. Oral micronized progesterone (150 mg) or placebo capsules were administered at 11:00 pm on the evening of admission and 2.5 hours before each cocaine session. The dose of progesterone was selected to mimic normal luteal phase progesterone levels. During each phase, cocaine self-administration sessions occurred at 9:00 AM and again at 1:00 PM on 2 consecutive days, for a total of 4 sessions. Prior to each session, participants were given $25 of their study earnings to purchase cocaine during the session. Participants sampled the dose available that session (either 0, 12, 25 or 50 mg cocaine base) and then had the opportunity to purchase up to 5 additional doses, at a cost of $5 per dose.
Results: To date 4 African-American women have completed the protocol; all had normal ovulatory menstrual cycles ranging from 26 to 30 days. During the normal follicular phase, mean estradiol levels were 79 pg/ml and progesterone levels were 0.69 ng/ml. During the normal luteal phase mean estradiol levels were 120 pg/ml and progesterone levels were 5.8 ng/ml. During the follicular phase that micronized progesterone was administered, mean estradiol levels were 84 pg/ml and progesterone levels were 5.7 ng/ml. The number of cocaine doses self-administered increased as a function of cocaine dose. However, exogenous progesterone significantly decreased self-administration of 12 and 50 mg cocaine compared to the follicular phase. Exogenous progesterone also decreased some of the subjective effects of cocaine, e.g., "Drug Quality" cluster scores during the follicular phase were decreased after 25 and 50 mg cocaine when progesterone was administered compared to the normal follicular phase. Cocaine produced dose-related increases in heart rate and blood pressure. Progesterone administration attenuated the cocaine-induced increases in heart rate, particularly after 25 and 50 mg smoked cocaine.
Discussion: These preliminary results are consistent with our previous findings and this study shows that exogenous progesterone also decreases cocaine self-administration. Supported by NIDA grant DA-08105 and NIH grant MOI-RR-00645.

39. Differences in the Dopamine System Correspond to Individual Differences in Pavlovian Conditioned Approach Behavior

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Sponsor: Terry Robinson

Background: The way an individual responds to its environment and to salient stimuli is a key determinant of vulnerability to psychopathology such as substance abuse. One theme common to the many theories of addiction is that the development of addictive behaviors depends upon experience with crucial environmental determinants conducive to the induction of the disorder. Perhaps this is because the neural circuitry regulating an organism's responses to its environment is the same circuitry underlying addictive behaviors. Therefore, in the present study we investigated individual differences and the neurobiological mechanisms underlying Pavlovian stimulus-reward learning in rodents.
Methods: To examine the ability of environmental stimuli to control behavior we studied Pavlovian conditioned approach (PCA) behavior using an autoshaping paradigm. The Pavlovian autoshaping procedures consisted of the brief presentation of an illuminated retractable lever (conditioned stimulus, CS) followed by the response-independent delivery of a food pellet (unconditioned stimulus, US). The topography of the Pavlovian conditioned response includes lever-CS directed approach, followed by grasping and gnawing of the lever, which is recorded as lever presses. Rats were categorized based on their Pavlovian conditioned approach (PCA) behavior. High lever pressers (HLP) were those animals that made rapid associations as demonstrated by PCA towards the lever (e.g. CS) while low lever pressers (LLP) did not develop PCA behavior, but spent more time attending to the food receptacle during presentation of the CS. Both groups of animals consumed all of the food pellets that were delivered during a training session. One training session consisted of 25 trials (CS-US pairings) presented on a variable interval schedule. Each session lasted approximately 40 minutes per day. We have previously shown that after the first day of training, we can predict with approximately 75% accuracy whether or not the animal will be classified as LLP or HLP at the end of the 5th day of training. Therefore, to examine the neurobiological mechanisms underlying the behavior described above, brains were obtained from high and low lever pressers immediately following the first and the fifth autoshaping sessions. A subset of control animals were sacrificed prior to the first training session (e.g. basal time point). Using in situ hybridization we examined the molecular expression of dopamine transporter (DAT) and tyrosine hydroxylase (TH) in the ventral tegmental area (VTA) of the mesolimbic dopamine system.
Results: If both LLP and HLP groups are combined, there is no effect of time (basal, Post-Day 1 or Post-Day 5) on DAT or TH mRNA levels. There were no differences between HLP and LLP in either DAT or TH expression following the first day of training. Interestingly, however, LLP showed increased expression of both DAT mRNA and TH mRNA relative to HLP following the 5th day of training. LLP also showed a slight increase in both DAT mRNA and TH mRNA following the fifth training session compared to the first.
Discussion: These findings suggest that the behavior resulting from a Pavlovian autoshaping paradigm is related to changes in the mesolimbic dopaminergic system, the same neural circuitry implicated in substance abuse. Further investigation of postsynaptic mechanisms is necessary to determine whether LLPs have increased or decreased dopaminergic tone relative to HLPs. Ongoing studies are also investigating how these phenotypes differ in drug-taking behavior and the induction of psychostimulant sensitization.

40. Differential Roles for the Basolateral Amygdala and Medial Prefrontal Cortex in Extinction and Cue-Induced Reinstatement of Food-Seeking Behavior

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Background: Reinstatement of previously extinguished instrumental responding for drug-related cues has been used as an animal model for relapse of drug taking, and has been shown to be disrupted by inactivation of either the basolateral amygdala (BLA) or the medial prefrontal cortex (mPFC) (McLaughlin and See, 2003). However, the role that these brain regions play in reinstatement of instrumental responding for conditioned reinforcers associated with natural rewards (i.e.; food) is currently unknown.
Methods: In the present study, rats with bilateral guide cannulae implanted into either the BLA or the prelimbic region of the mPFC were trained to press one of two levers to receive delivery of food reward paired with a complex light/tone conditioned stimulus (CS). Following 5 days of training on a variable-ratio schedule 5 (VR5), they underwent 3-6 days of extinction of lever pressing, where no CS or food was delivered, until they achieved a criterion of <10% of their baseline responding on the VR5 schedule. Reinstatement of extinguished lever pressing was measured during response-contingent presentations of the CS alone. Rats received intracranial infusions of either saline or the local anesthetic bupivacaine prior to reinstatement test days. In a separate series of experiments, rats received intracranial in-

fusions of either saline or bupivicaine prior to the first day of extinction training, after which they received daily extinction trails with no infusions.
Results: Following control infusions into the BLA, rats displayed a significant increase in lever pressing (~100%) during reinstatement sessions relative to their last day of extinction training. Contrary to expectations, inactivation of the BLA did not attenuate responding for the CS in the absence of food delivery. Instead, BLA inactivation significantly enhanced responding relative to vehicle control treatments (~400% increase in responding relative to their last day of extinction training.). Analysis of within-session responding revealed that BLA inactivation retarded extinction of lever pressing in response to the CS in the absence of food delivery. In addition, inactivation of the BLA prior to the first day of extinction training (i.e.: no food or CS delivery) disrupted extinction of lever pressing on the subsequent training session relative to animals receiving saline infusions. In contrast to the above mentioned findings, inactivation of the mPFC did not reliably effect either cue-induced reinstatement or extinction of lever pressing.
Discussion: When viewed collectively, these data suggest that neural circuits which underlie cue-induced reinstatement for drug-related stimuli are different from those which mediate responding for conditioned reinforcers associated with natural rewards. Moreover, they demonstrate that the BLA plays a role in facilitating the extinction of instrumental responding for both primary and conditioned reinforcement. This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health, and was supported by a grant from the Canadian Institutes of Health Research.

41. The Role of the Basolateral Amygdala in Consolidation of Stimulus-Reward Associations and Subsequent Conditioned Cued-Reinstatement of Cocaine Seeking
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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Consolidation of drug-conditioned stimulus (CS) associations into long-term memories is necessary for long-lasting CS control over drug seeking and the basolateral amygdala (BLA) likely plays a role in this phenomenon.
Methods: To test this hypothesis, rats were first trained to lever press for cocaine infusions in the absence of an explicit CS (2 h/session, 5 days). Rats then underwent a 1- or 2-h Pavlovian classical conditioning (CC) session during which they received passive cocaine-CS (light+tone) pairings while the levers were retracted. During the 2-h CC session, the number of pairings equaled the mean number of cocaine infusions earned on the preceding two self-administration days (2-2 CC session). During the 1-h CC session, the number of pairings equaled the mean number of cocaine infusions earned during the first 1 h (1-1 CC session), or during 2 hs (1-2 CC session), of self-administration. Immediately after the CC session, rats received an intra-BLA microinfusion of vehicle or tetrodotoxin (TTX; 5ng/side), a Na+ channel blocker that reversibly impairs neuronal activity. Rats then lever pressed for cocaine infusions in the absence of the CS (5 days) and underwent extinction training (min. 7 days). After responding extinguished, reinstatement of lever pressing was assessed in the response-contingent presence of the CS.
Results: Rats that had received vehicle immediately after the 2-2 or 1-1 CC sessions subsequently exhibited significant conditioned cued-reinstatement relative to extinction, whereas rats that had received vehicle after the 1-2 CC session did not reinstate. Rats that had received TTX into the BLA immediately after the 2-2 or 1-1 CC sessions did not reinstate relative to extinction.
Discussion: These findings support the hypothesis that the functional integrity of the BLA is necessary for the consolidation of drug-CS memories that, in turn, activate cocaine seeking during relapse. Understanding the neural mechanisms of cue memory consolidation may provide information toward the development of effective treatments for cocaine dependence. Support Contributed By: NIDA P50 DA015369, NIDA R01 DA10462, and NIDA R01 DA017673.

42. Regulation of Alcohol-Heightened Aggression by Dorsal Raphe and Prefrontal Cortical 5-HT1B Receptors: Evidence from Microinjection and Microdialysis Studies in Mice
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Background: A significant minority of humans, macaques and rodents engage in escalated levels of aggression after consumption of a moderate dose of alcohol. 5-HT1B receptor agonists may be important modulators of heightened aggression because systemic administration of the 5-HT1B agonist CP-94,253 decreases alcohol-heightened aggression (AHA) with greater potency relative to alcohol non-heightened aggression (ANA) without sedation. Serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) project primarily to the prefrontal cortex (PFC) and striatum where 5-HT1B receptors are pre-and post-synaptically located. The current objective was to characterize the respective roles of somatodendritic and post-synaptic 5-HT1B receptor populations in the DRN versus the orbitofrontal (OFC) and infralimbic (ILC) cortex on the modulation of aggressive behavior and extracellular levels of 5-HT.
Methods: Male mice were trained, on a panel placed into their home cage, to self-administer ethanol (EtOH) via sucrose fading by performing an operant response that was rewarded with a delivery of 6% EtOH. Each mouse was repeatedly tested for aggression after consumption of either 1.0 g/kg EtOH or water, and was characterized as AHA or ANA based on these confrontations. Next, a cannula was implanted into either the DRN, OFC or ILC. These mice were subsequently tested for aggression after drinking either 1.0 g/kg EtOH or water prior to a 0.5 l infusion of CP-94,253, zolmitriptan or (+) 8-OH-DPAT. In order to assess the effects of CP-94,253 on cortical extracellular levels of 5-HT, an additional group of mice were implanted with a guide cannula into the ILC. One week later, a 2 mm microdialysis probe was inserted and 1 uM CP-94,253 was perfused through the probe. Twenty minute samples were collected before, during and after reverse microdialysis.
Results: Forty percent of the mice were more aggressive after drinking EtOH, confirming the aggression- heightening effects of EtOH. Microinjection of both CP-94,253 and (+) 8-OH-DPAT into the DRN after self-administration of both water or EtOH decreased aggression, replicating the behavioral effects observed after systemic administration of both ligands. In contrast, microinjection of 1 ug CP-94,253 into both regions of the cortex increased aggressive behavior only after self-administration of 1g/kg EtOH. Reverse microdialysis of CP-94,253 into the ILC increased extracellular levels of 5-HT for the 40 min duration of drug perfusion and subsequently levels 5-HT decreased during recovery. Interestingly, this increase was blunted when the reverse perfusion occurred after EtOH self-administration.
Discussion: Direct application of 5-HT1B agonists into the prefrontal cortex produces increases in aggressive behavior and extracellular 5-HT rather than decreases, as seen after systemic administration. This pattern of results suggests that the attenuation of aggressive behavior relies on 5-HT1B receptor pools other than those in the ILC.

43. Evidence of White Matter Pathology in First-Episode Manic Adolescents with Bipolar Disorder: A Diffusion Tensor Imaging Study
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Background: Several lines of evidence suggest that bipolar symptomatology involves a dysconnectivity syndrome marked by subtle neuropathologic changes in white matter tracts. Our previous diffusion

tensor imaging (DTI) findings support these suggestions and indicate the presence of axonal disorganization in the prefrontal cortex of patients with bipolar disorder, reflected in decreased fractional anisotropy (FA) without significant changes in the trace apparent diffusion coefficient (TADC). At least one subsequent study however, found evidence for changes in TADC in a cohort of older bipolar patients, with a longer duration of illness. Together, these studies suggest the presence of developmental abnormalities relatively early in the course of bipolar disorder with possible neuropathic changes appearing over time. The chronic nature of the patients studied however, has limited interpretation of DTI findings. In this study we utilized DTI to study white matter tracts of first-episode adolescent bipolar patients, to address whether white matter abnormalities are an innate feature of bipolar disorder, and to determine the nature of these abnormalities at the start of bipolar symptomatology.
Methods: Eleven medication-naive, first-episode bipolar adolescents and seventeen healthy controls received high-resolution structural and DTI scans, from which were derived fractional anisotropy (FA) and trace apparent diffusion coefficient (TADC) maps. FA and TADC of a priori determined prefrontal and posterior parietal regions-of-interest, adjacent to areas in which we have previously observed neurofunctional changes, were compared across groups. In addition, FA and TADC maps were normalized, and exploratory voxel-by-voxel analyses were performed.
Results: Bipolar adolescents showed significantly decreased FA in superior frontal white matter tracts ($t=2.84$, $df=26$, $p=0.009$). Differences in FA were also observed on the voxel-by-voxel analysis, in portions of the right prefrontal white matter tracts. TADC significantly differed between groups only in the voxel-based analysis, in portions of the left occipital white matter.
Discussion: These findings suggest that white matter abnormalities are non-uniformly present early in the course of bipolar disorder and may consist largely of axonal disorganization, particularly in the frontal cortex. Observing these changes in young first-episode patients further suggests that white matter pathology may represent an early marker of bipolar disorder.

44. Quetiapine Efficacy in Bipolar Adolescents with Depressive Symptoms

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Background: Bipolar disorder is a major psychiatric illness associated with significant morbidity and mortality. In adolescents, bipolar disorder may be responsible for impaired social, cognitive and psychological function. Although the majority of bipolar studies in adolescents have focused on manic episodes, depression is a common presenting symptom and may be responsible for much of the morbidity associated with bipolar disorder in these patients. Moreover, treatment of depression in bipolar adolescents is complicated by a lack of efficacy data. The objective of this analysis was to combine data from three prospective studies to investigate the efficacy of quetiapine for reducing depressive symptoms and suicidal ideation in adolescents diagnosed with bipolar disorder, and adolescents with a strong familial risk for developing bipolar disorder who have been diagnosed with another mood disorder.
Methods: Three studies measured change in depressive symptoms including suicidal ideation following treatment with quetiapine in adolescents meeting DSM-IV criteria for bipolar disorder or adolescents with a mood disorder and a family history of bipolar disorder (total N=65). In study 1, 30 adolescents with bipolar disorder were treated with quetiapine and divalproex (N=15) or divalproex alone (N=15) for 6 weeks in a double-blind, placebo-controlled study of quetiapine as adjunctive treatment to divalproex. In study 2, 50 adolescents received quetiapine (N=25) or divalproex (N=25) monotherapy for 4 weeks in a double-blind, randomized, controlled study. In study 3, 25 adolescents with a mood disorder (major depressive disorder, bipolar disorder, types I, II or NOS) and a parent with bipolar disorder received quetiapine monotherapy for 12 weeks in a single-blind (rater-blind), prospective study.
Results: Quetiapine (mean 423 mg/day) combined with divalproex reduced mean Childrens Depression Rating Scale (CDRS) score from 50 to 24 over 6 weeks in 15 patients with a mixed or manic episode ($p<0.0001$), a significantly greater decrease than observed with divalproex alone ($p<0.004$). Quetiapine monotherapy (mean 412 mg/day) for 4 weeks decreased mean CDRS score from 52 to 25 in 25 patients with a mixed or manic episode ($p<0.0001$). Quetiapine monotherapy (mean 447 mg/day) for 12 weeks decreased mean CDRS score from 40 to 29 in 25 patients with a major depressive episode and a parent with bipolar disorder ($p<0.0001$). The mean CDRS suicidality item score decreased significantly ($p<0.0001$) between baseline and endpoint from 3.0 to 1.5 in the 65 adolescents with an episode of depression and from 4.4 to 1.7 in the 38 patients with a CDRS suicide item score >1 at baseline.
Discussion: As was observed in studies of bipolar adults with depressive symptoms, quetiapine effectively treats symptoms of depression in adolescents with or at familial risk for developing bipolar disorder. Changes in the CDRS suicidality item score suggest that quetiapine may improve suicidal ideation in these patients. However, placebo-controlled studies to confirm these findings in larger samples are necessary.

45. Measurement of Brain Betabolites in Type 2 Diabetes and Depression Using Proton Magnetic Resonance Spectroscopy

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Sponsor: Past Travel Awardee, NIMH, 2003

Background: Type 2 diabetes and major depression are disorders that are mutual risk factors and may share similar pathophysiological mechanisms. To further understand these shared mechanisms, the purpose of our study was to examine the biochemical basis of depression in patients with type 2 diabetes using proton MRS.
Methods: Patients with type 2 diabetes and major depression (n=20) were scanned along with patients with diabetes alone (n=24) and healthy controls (n=21) on a 1.5 T MRI/MRS scanner. Voxels were placed bilaterally in dorsolateral white matter and caudate nuclei, both regions important in the circuitry of late-life depression. Absolute values of myo-inositol, creatine, N-acetylaspartate, glutamate, glutamine, and choline were measured using the LC-Model algorithm.
Results: Glutamine concentrations in depressed diabetic patients were significantly lower ($p<.05$) in the left caudate by 25.5% and in the right caudate by 22.4% as compared to healthy controls. Glutamate concentrations in the right caudate of diabetic depressed subjects were significantly lower ($p < .05$) as compared to healthy controls by 15.9%. Myo-inositol concentrations were significantly increased ($p<.05$) in diabetic controls and depressed diabetic patients, by 30% and 21% respectively, in right frontal white matter as compared to healthy controls.
Discussion: Alterations in glutamate and glutamine levels in subcortical regions along with white matter changes are associated with major depression in patients with type 2 diabetes.

46. Escitalopram Responders Have Higher Quality of Life Scores Than Placebo Responders

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Background: Quality of life (QOL) has been shown to be higher in panic disorder patients responding to an antidepressant than to placebo. We hypothesized that patients with Generalized Anxiety Disorder (GAD) responding to escitalopram would have higher QOL scores than those responding to placebo.

Methods: Data were pooled from three similarly designed, randomized, placebo-controlled, 8-week, flexible-dose studies of escitalopram (10 to 20 mg/day) in patients with GAD (escitalopram N=421, placebo N=419). Clinical response was defined as a ≥ 50% decrease from baseline in HAMA scores or a Clinical Global Impression Improvement (CGI-I) score of ≤ 2 at endpoint. QOL was assessed by the Quality of Life Enjoyment and Satisfaction Questionnaire global score.
Results: There were no statistically significant differences in baseline characteristics, including QOL measures and HAMA scores, for escitalopram responders compared with placebo responders. For CGI-responders (escitalopram N=219, placebo N=157), mean changes in QOL scores were 9.9 and 6.6, respectively (LOCF, p=0.003). For HAMA responders (escitalopram N=200, placebo N=120), mean changes in QOL were 10.2 and 6.8, respectively (LOCF, p=0.009). This effect did not appear to be dependent on outcomes in HAMA scores.
Discussion: Escitalopram-responders showed significantly greater improvement in QOL scores compared with placebo-responders. These results suggest that response to escitalopram is qualitatively different than a placebo response.

47. Gender Differences in Sleep Regulation in Depressed Adults and Healthy Controls

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Background: Major depressive disorders (MDD) are strongly associated with sleep disturbances and abnormalities in the timing of REM and NREM sleep cycles. Reduced slow-wave activity amplitude (SWA), delta in NREM sleep, has also been reported, primarily in men with MDD. It is not clear whether reduced SWA results from abnormalities in sleep regulatory mechanisms or simply a reduction in synchronous neural activity. The regulation of SWA is under tight homeostatic control in healthy individuals such that the amount of SWA after sleep onset is directly proportional to the amount of prior wakefulness. The time course of SWA is well-known, with peak SWA in the first NREM sleep episode and rapid dissipation over subsequent NREM sleep time. If sleep regulation was impaired in men with MDD, it would be reflected in either lower accumulation or a slower dissipation of SWA across NREM sleep (or both). The present study tested the hypothesis that reduced SWA in MDD results from impairment in basic homeostatic sleep regulation that is gender-specific.
Methods: 80 subjects, 20-40 years of age participated in study. Half met DSM-IV-R criteria for MDD and were symptomatic and unmedicated at the time of study. 40 age- and gender-matched healthy normal controls (NC) were also included. All participants kept an 11pm-6am sleep schedule for 5 days at home, verified by actigraphy and sleep diary, followed by 3 consecutive nights in the Sleep Lab. Night 1 served as laboratory adaptation and baseline EEG measures were collected on night 2. Night 3, served as the homeostatic sleep challenge night, extending prior wakefulness for an extra 3 hours with bedtime at 2 am and rise time at 9 am. Note, that total available sleep time was held constant at 7 hours throughout the study. EEG activity was recorded from right and left frontal, central, parietal and occipital electrodes and digitized at 256 Hz. Power spectral analysis quantified sleep EEG in the 0.5-3.9 Hz frequency band. Visual sleep stage scoring was conducted according to standard criteria. Power spectral data were averaged across 30 s epochs to be comparable to sleep stage scoring and coded for NREM period identifying SWA and coded for gender and group (MDD vs NC). Two measures of SWA were evaluated statistically, SWA power in each NREM period on baseline and challenge nights, and SWA on the challenge night expressed relative to baseline SWA. Repeated-measures MANOVA evaluated between-group differences, testing the gender by group interaction first. Exponential regression analyses evaluated the time course of SWA and were compared across groups.
Results: Significant group by gender interactions were evident. As hypothesized, men with MDD failed to show an enhancement of SWA in response to sleep challenge. Accumulation of SWA was significantly lower than all other groups with a more shallow dissipation over the night. Gender differences in the MDD group were 2-3 times larger than those observed in healthy controls. SWA did not differ between healthy men and women at baseline, but did after the sleep challenge. NC women showed a larger response to sleep delay than did NC men.
Discussion: These results indicate that sleep regulation is impaired in MDD, but is strongly influenced by gender. The findings provide strong support that basic sleep homeostasis is impaired in depressed men and that the pathophysiology of depression differs in men and women. These results also indicate that gender differences in sleep in healthy individuals are only evident under challenge conditions. Taken together, the findings indicate that sleep regulation is disease and gender dependent.

48. Glutamate Transporter Gene (SLC1A1) Associated with Obsessive Compulsive Disorder

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Sponsor: Past Travel Awardee, ADAA, 2004

Background: There is strong evidence from family and twin studies that genetic determinants play an important role in the etiology of obsessive-compulsive disorder (OCD). In the only genome scan to date of OCD, suggestive linkage was reported to the chromosomal region 9p24, a finding that was subsequently replicated. This region contains the gene encoding the neuronal glutamate transporter, SLC1A1. SLC1A1 represents an excellent functional candidate gene for OCD based on evidence from neuroimaging and animal studies that altered glutamatergic neurotransmission is implicated in the pathogenesis of this disorder. Based on this rationale, we conducted a candidate gene association study to determine whether sequence variants in SLC1A1 were associated with transmission of the OCD trait.
Methods: Participants included 157 Caucasian probands with DSM-IV OCD recruited from consecutive referrals to a specialized anxiety disorders outpatient clinic and their first-degree relatives (476 individuals in total). Nine single nucleotide polymorphisms (SNPs) spanning SLC1A1 were genotyped. Single locus and haplotype analyses were performed using the Family Based Association Test (FBAT) and the Transmission Disequilibrium Test (TDT). Traits examined included DSM-IV OCD diagnosis and highest lifetime symptom severity as measured using the Yale-Brown Obsessive-Compulsive Scale (YBOCS).
Results: After correction for permutation testing, two variants - rs301434 (χ^2= 12.04, p = 0.006) and rs301435 (χ^2= 9.24, p = 0.03) - located within a single haplotype block were found to be associated with transmission of OCD. Furthermore, a specific two marker haplotype within this block was significantly associated with OCD (χ^2 = 12.60, p = 0.005). This haplotype association was observed in statistically significant in transmissions to male (χ^2 = 9.39, p = 0.02) but not female offspring.
Discussion: Our results provide evidence that sequence variation in SLC1A1 may be associated with susceptibility to OCD, particularly in males. Further support for this finding comes from an independent report of an association between early onset OCD and SLC1A1 which is strongest in males (Dickel et al., submitted). These results also provide support for the role of altered glutamatergic neurotransmission in the pathogenesis of OCD.

49. A Study of Long-Acting, Injectable Risperidone in Frequently Relapsing Bipolar Disorder: Preliminary Findings

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Sponsor: Deborah Yurgelun-Todd

Background: Many patients with bipolar disorder (BD) relapse frequently, demonstrating significant morbidity and poor long-term

outcomes. This ongoing trial identifies a population of BD subjects based on their unstable clinical course and poor outcomes, and evaluates adjunctive treatment with long-acting injectable (LAI) risperidone on mood symptom control and functioning. We discuss overall study design and preliminary findings from the open-label (OL) study phase.
Methods: Patients aged 18 to 70 years meeting DSM-IV criteria for BD who experienced ≥4 episodes requiring clinical intervention in the past 12 mo and ≥2 episodes in the past 6 mo, received OL augmentation of their treatment-as-usual with LAI risperidone (25-50 mg) for 16 wk. Remitters (Young Mania Rating Scale [YMRS] and Montgomery-Asberg Depression Rating Scale [MADRS] ≤10 over the last 4 wk of OL phase) were eligible to be randomized to placebo or their OL-phase LAI risperidone dosage in a double-blind (DB), 52-week, relapse-prevention phase. Depressive and manic symptoms were rated by MADRS and YMRS, respectively, and clinical status by Clinical Global Impressions of Severity (CGI-S).
Results: Preliminary OL-phase results for the first 84 subjects follow: 74 (88%) had bipolar I disorder; 10 (12%) had bipolar II disorder. The mean age (±standard deviation [SD]) was 41.3 ± 11.9 years, and 42 (50%) were women. Eighty (95%) had received ≥3 months' exposure to lithium, an antiepileptic, or an atypical antipsychotic within the previous 2 years. Fifty-five percent had ≥3 lifetime hospitalizations; the mean (±SD) number of hospitalizations was 6.9 ± 10.6. Forty-six percent had a lifetime suicide attempt (mean [±SD] number of attempts 1.4 ±2.4); 14% had attempted suicide within the past 2 years; 32 (38%) had a lifetime history of substance abuse. At baseline, 54 (64%) of the 84 subjects were moderately or markedly ill by the CGI-S; 37% scored ≥20 on the YMRS; 38% scored ≥20 by MADRS. Mean (±SD) YMRS and MADRS scores at study entry were 15.7 ± 10.9 and 12.7 ± 11.3, respectively. Sixty-two subjects (69%) completed the OL phase; 41 (50%) achieved remission criteria, thus eligible to enter the DB phase; 21 (23%) failed to meet remission criteria but chose to continue adjunctive treatment with OL LAI risperidone. Reasons for discontinuation from the OL phase included (n [%]): adverse events (5 [6%]); lost to follow-up (1 [1%]); noncompliance (1 [1%]); protocol violation (1 [1%]); withdrawal of consent (14 [17%]). Those with CGI-S scores of moderately ill or worse decreased from 64% at baseline to 19% at OL endpoint. Mean change scores (±SD) for YMRS and MADRS were -10.4 ± 11.3 ($P < 0.001$) and -4.5 ± 12.6 ($P < 0.05$) at OL endpoint, respectively.
Discussion: This study was designed to evaluate the efficacy of maintenance treatment with adjunctive LAI risperidone to prevent relapse of mood disorder in subjects with frequently relapsing BD (FRBD). Preliminary OL findings suggest adjunctive treatment with LAI risperidone may reduce symptoms for patients with FRBD who suffer frequent mood episodes requiring clinical interventions despite currently available treatments. Conclusions about the role of LAI risperidone for acute treatment of FRBD are limited by the absence of a placebo comparator in the OL phase. Additional analyses will be presented to investigate the time course and stability of symptom improvement with LAI risperidone. Source of Funding: Janssen, L.P.

50. Association of an Ancient CRH Gene Haplotype with HPA Axis Activity in Rhesus Macaques
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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Corticotropin-releasing hormone (CRH) is the primary neuropeptide responsible for activation of the hypothalamic-pituitary-adrenal (HPA) axis. Perturbation of both the extrahypothalamic/hypothalamic CRH systems and the HPA axis are associated with certain neuropsychiatric disorders, in addition to alcohol use and withdrawal. As such, the CRH gene may be a good candidate for investigating genetic variation as it relates to vulnerability to anxiety and mood disorders, neuroendocrine stress axis dysregulation, and alcoholism. We have identified a number of polymorphisms within the 5 'flanking and coding regions for the CRH gene (rhCRH) in rhesus macaques. Among these is a snp within a putative glucocorticoid response element (GRE) half-site. This snp is in allelic identity with 18 other polymorphisms (rhCRH-A2) and is present in 15% animals tested in 4 different populations of rhesus macaques. We wanted to test whether the −2232C>G snp would result in diminished GR binding, leading to glucocorticoid resistance of the rhCRH promoter. We also wanted to determine whether the rhCRH-A2 haplotype would be associated with increased HPA axis activity at baseline, following a psychosocial stressor and in response to alcohol.
Methods: At 6 months of age, rhesus infants (N=232) were subjected to 96h of social separation stress, and adrenocorticotropin (ACTH) and cortisol levels were determined at baseline and at 1, 2 and 96 h of separation stress. At approximately 4 y of age, a subset of alcohol-naive animals (N=90) received an intravenous infusion of alcohol (2-2.2 g/kg), and ACTH and cortisol levels were determined following alcohol infusion. Effects of rhCRH gene variation on HPA axis output were analyzed using repeated measures ANOVA.
Results: Among infants, rhCRH-A2 was associated with increased ACTH levels, but only in the absence of stress. Among adult animals, the rhCRH-A2 haplotype cluster was also associated with increased ACTH at baseline and higher ACTH and cortisol responses to alcohol.
Discussion: These data demonstrate that rhCRH gene variation is associated with increased HPA activity both at baseline and in response to alcohol in rhesus macaques and may suggest a role for human CRH gene variation in the susceptibility to stress- and alcohol-related disorders.

51. Regional Cerebral Metabolism and Anxiety Symptoms in Bipolar Depression: Effects of Levothyroxine
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Sponsor: Peter Whybrow

Background: Adjunctive therapy with supraphysiological doses of levothyroxine has emerged as a novel approach for patients receiving antidepressants and mood stabilizers for refractory bipolar disorders. This study examined the relationships 1) between regional cerebral glucose metabolism and the severity of state and trait anxiety symptoms and 2) between changes in anxiety symptoms and changes of relative regional activity in bipolar depressed patients receiving adjunctive treatment with levothyroxine.
Methods: Regional brain activity was assessed with positron emission tomography and [18F]fluorodeoxyglucose in 10 euthyroid depressed women with bipolar disorder before and after 7 weeks of adjunctive therapy with supraphysiological doses of levothyroxine (mean dose 320 mcg/day). The primary biological measures were relative regional radioactivity (taken as a surrogate index of glucose metabolism) in pre-selected brain regions. Relationships were assessed between changes in regional (relative to global) activity and changes in state and trait anxiety as measured with the State-Trait Anxiety Inventory.
Results: Before levothyroxine treatment, symptoms of trait and state anxiety covaried positively with relative brain activity bilaterally in the left dorsal anterior cingulate cortex, superior temporal gyrus, insula, parahippocampal gyri, amygdala and hippocampus, and the left ventral striatum. In addition, state anxiety covaried positively with relative activity in the cerebellar vermis. Levothyroxine treatment improved anxiety symptoms significantly; the reduction in anxiety scores was significantly correlated directly with decreases in relative

activity of the right hippocampus and left parahippocampal gyrus (trait anxiety) and of the hippocampus (bilaterally), right parahippocampal gyrus and left thalamus (state anxiety). After treatment, state anxiety correlated inversely with relative activity in the right subgenual cingulate cortex, the left middle frontal cortex, and bilaterally with the left dorsal anterior cingulate cortex (state and trait anxiety).
Discussion: Co-morbid anxiety symptoms have specific regional cerebral metabolic correlates that improve with levothyroxine treatment in bipolar depression.

52. Duloxetine: Meta-Analyses of Suicidal Behaviors and Thoughts in Clinical Trials for Major Depressive Disorder
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Sponsor: Ross Baldessarini

Background: Given uncertain relationships between antidepressant treatment and suicidality we systematically reviewed data on suicide-related behaviors and ideation from Phase II and III placebo- (PBO) controlled clinical trials of duloxetine (DLX) for major depressive disorder (MDD) supported by Eli Lilly & Co. and Shionogi Co. Ltd.
Methods: We searched databases of all PBO-controlled trials of DLX in MDD (completed by 02/04) for suicide-related events, using computerized text-string searches of both adverse event reports and investigator comments in study case reports forms. Suicide-related phenomena were categorized as: [1] completed suicide, [2] non-fatal suicide attempt, [3] aborted suicidal act, [4] instrumental suicidal act or (suicidal gesture), or [5] suicidal ideation. We compared the incidence of these phenomena during treatment with DLX vs. PBO, using Mantel-Haenszel Incidence Difference (MHID) and exposure time-adjusted rate difference (MHRD) methods. We also assessed changes on Item-3 (suicidality) of the 17-item Hamilton Rating Scale for Depression (HAMD17) for worsening/improvement of suicidal ideation and emergence of substantial suicidal ideation.
Results: Data from 12 trials involved 2996 patients (1812 given DLX, 1814 PBO). There were no statistically significant differences in the incidence of suicide-related measures with DLX vs. PBO. The MHID for suicide-related behaviors (Categories 1—4) was —0.03 [95% CI: —0.48 to 0.42], for fatal and non-fatal suicide attempts (Categories 1—2) 0.01 [95% CI: —0.41 to 0.42], and for suicide-related thoughts and behaviors overall (Categories 1—5) 0.20 [95% CI: —0.70 to 1.10]. Respective exposure time-adjusted rate differences (MHRD) were —0.002 [95% CI: —0.02 to 0.02] for behaviors, —0.0003 [95% CI: —0.02 to 0.02] for attempts, and 0.009 [95% CI: —0.03 to 0.05] overall. Changes in HAMD-Item-3 scores showed more worsening of suicidal ideation with PBO (MHID: —4.25, 95% CI: —6.55 to —1.95; $p\langle 0.001$) and more improvement with DLX (MHID: 9.56, 95% CI: 4.50 to 14.6; $p\langle 0.001$). Seven (0.58%) DLX- and 2 (0.24%) PBO-treated patients had emergence of substantial suicidal ideation (Item-3 change from 0 or 1 to ≥3; p=0.090); 3 (0.52%) DLX and no PBO patients increased from 0 to 3 (p=0.066); 12 (2.07%) DLX and 12 (2.88%) PBO patients increased from 0 to 2 (p=0.44), and none changed from 0 to 4.
Discussion: We found no difference between DLX and PBO in measures categorized as suicide-related behaviors or ideation. Indeed, HAMD-Item-3 score (suicidality) changes indicated more improvement and less worsening of suicidal ideation with DLX than PBO. Although suicide-related behaviors were uncommon, we found no evidence overall of an increased risk of suicidal acts or suicidal ideation during treatment with DLX compared to PBO.

53. Generalized Anxiety and Pain: Associations and Implications
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Sponsor: Past Travel Awardee, ECNP-ACNP Fellow, 2004

Background: The reciprocal relationships between symptoms of pain, anxiety and depression as well as some implications for therapeutic management are well known for a long time. Little is known, however, about the association between pain and pain disorder on the one hand and specific mental disorders and Generalized Anxiety Disorder (GAD) in particular on the other hand. The aims of this paper are to examine (a) whether generalized anxiety is associated with clinically significant pain symptoms/disorder, (b) whether this association persists after controlling for potential confounders, focusing on depression, and (c) whether GAD-pain affects functional outcomes.
Methods: Nationally representative community sample of N = 4,181 participants aged 18-65 years. Mental disorders were assessed using a fully standardized diagnostic interview (M-CIDI) and DSM-IV criteria. Several thresholds were used to define GAD and clinically significant pain. Associations (Odds Ratios, OR) were revealed using multinomial logistic regression models.
Results: (a) GAD and pain are significantly associated. All symptom and threshold expressions of GAD are associated with pain symptoms and pain disorder with some evidence for a dose-response relationship as indicated by increasing Odds Ratios towards DSM-IV GAD and pain disorder (OR range: 1.7 - 15.0). Although sex, age, major depression, and dysthymia all have an independent influence, the associations between GAD and pain remain significant in a multiple model, except for attenuated OR`s in subthreshold GAD. Implications of the co-occurrence of GAD and pain include a forward shift in symptom onset as well as increased odds for adverse outcomes in terms of disability, quality of life, and service utilization.
Discussion: The association of generalized anxiety and pain is not artefactual and has important public health implications. The epidemiological findings provide some indirect evidence that both conditions might share similar neurobiologic mechanisms. This is also consistent with the observation of beneficial effects of various psychopharmacological agents on both GAD and pain, such as SNRIs, GABAergic as well as more recently non-GABAergic compounds.

54. Brain Regional α-[11C]Methyl-L-Tryptophan Trapping in Medication-Free Patients With Obsessive Compulsive Disorder
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Sponsor: Theodore Sourkes

Background: The hypothesis of a central sertonin (5HT) dysfunction in Obsessive Compulsive Disorder (OCD) stems largely from pharmacological treatment studies, where enhancement of 5HT neurotransmission is thought to be critical for treatment efficacy. However, studies of the 5HT sytstem in OCD including measures of peripheral markers, pharmacological challenge studies, and more recent neuroimaging of central 5HT transporter leave us with inconsistent findings and no clear picture as to the specific abnormalities of the 5HT system in OCD.
Methods: Direct measures of central 5HT metabolism has recently been possible through Positron Emission Tomography (PET) coupled with the a-[11C]-methyl-L-tryptophan (11C-aMtrp) tracer, believed to provide an index of the brain regional rates of 5-HT synthesis, through the measure of 11C-aMtrp brain trapping constant K*. In this study the 11C-aMtrp method was applied to compare medica-

tion-free patients with current OCD (n=21) to normal controls (n=21) matched for age and gender. The functional PET data were analyzed with Statistical parametric mapping (SPM99). Pairwise t-statistical maps were obtained comparing OCD patients to controls; the regions considered statistically significant had an extended threshold of 100 voxels with a peak threshold of p=0.005. SPM was also applied to identify regions where a-MTrp trapping correlated with the scores of the Yale-Brown Obsessive-Compulsive Scale (YBOCS) collected at the time of scan.
Results: 21 OCD patients (15 men: mean ±SD age, 41 ± 11 years; and 6 women: mean ±SD age, 41 ±11 years) and 21 controls (15 men: mean ±SD age, 37 ± 15 years; and 6 women: mean ±SD age, 32.5± 9.9 years) participated in the study. There was no significant group effect in the whole sample, or in either sex analysed separately for global K* values (p=0.66). In the sample as a whole, a-[11C]MTrp trapping was significantly higher for subjects with OCD in the right hippocampus and the Left Temporal Gyrus (BA 20). There were no regions with significant decreases in OCD subjects compared to controls. A region of interest (ROI) based analysis confirmed the SPM findings. In addition the ROI analysis conducted in males (n=15) revealed a main group effect in the caudate nucleus, with significantly higher regional trapping ($p<0.05$) in OCD subjects. No volumetric differences were found for any of the ROI between OCD subjects and healthy controls. In the sample as a whole YBOCS total score correlated positively with a-[11C]MTrp trapping in the right middle and superior Temporal Gyrus (BAs 21, 38) and in the left inferior and middle Temporal Gyrus (BAs 20, 21). In OCD males (n=15), YBOCS score and a-[11C]MTrp trapping correlated positively in the right caudate, whereas in OCD females (n=6) significant positive correlations were seen in the left cuneus (BA 19), the right inferior and middle Temporal Gyrus (BA 21) and the right inferior and middle Frontal Gyrus (BA 47).
Discussion: These findings add to the cumulative evidence supporting serotonergic dysfunction in OCD, and more specifically a possible increase in brain regional 5HT in the hippocampus, the Temporal Gyrus and the Caudate nucleus.

55. Antidepressant Drug Combination from Treatment Initiation to Improve Therapeutic Response in Major Depression

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Sponsor: Pierre Blier

Background: The treatment of depression with a single drug is not always time efficient because only about 50% of patients achieve remission with a first agent, given at an adequate dose for a sufficient time. In addition, a second medication, usually a benzodiazepine, is often used to manage the most cumbersome symptoms. In the present study, two antidepressants were used from treatment initiation; they had complementary mechanisms of action on the serotonin and norepinephrine systems and side effect profiles that would tend to cancel each other out.
Methods: Mirtazapine (30 mg at bedtime) was given with either fluoxetine (20 mg/day), bupropion (150 mg/day), or venlafaxine (75 mg/day X 1 week, then 150 mg/day X 1 week, and 225 mg/day thereafter), with the latter three drugs being taken in the morning. The control treatment consisted of fluoxetine 20 mg/day. This was a six-week double-blind randomized trial. The patients who achieved remission were entered into a 6 month prolongation whereby one drug was discontinued if the patients were on two agents: mirtazapine was stopped abruptly in the patients who received fluoxetine, bupropion in the patients who took it with mirtazapine, and venlafaxine was tapered over two weeks.
Results: The preliminary results (using the Montgomery-Asberg Depression Rating Scale; MADRS) on the first 87 patients show a significant change from baseline at day 4 onward for the three combinations, whereas this occurred at day 7 in the fluoxetine group. The visit at which the response criterion was achieved (a mean 50% drop in the MADRS) was Day 28 in the fluoxetine group, Day 21 in the fluoxetine + mirtazapine group, and Day 14 in the bupropion + mirtazapine and the venlafaxine + mirtazapine groups. The number of patients achieving remission (HAM-D of 7 or less) were higher in the combination groups than in the fluoxetine alone arm: 7/22 in the fluoxetine group, 12/23 in the fluoxetine + mirtazapine, 11/20 in the bupropion + mirtazapine group, and 13/22 in the venlafaxine + mirtazapine arm. About half of the patients on the combinations relapsed after one drug was discontinued, most of them within one month. Adverse events were minimal and the dropout rate was less than 15%.
Discussion: In summary from these preliminary analyses, the combinations were well tolerated, appeared to work faster, and more effective than monotherapy. The final data set from the total sample of 100 patients will be presented at the meeting.

56. Subjective Evaluation of the Long-Term Cognitive Effects of Electroconvulsive Therapy

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Sponsor: Harold Sackeim

Background: Using highly structured assessments of meta-cognition, several recent studies have shown that a majority of patients rate their memory as improved following ECT. These subjective evaluations bear little relation to objective memory impairment, but are strongly associated with change in depressive symptoms. However, no study has assessed the effects of ECT on simpler, more global assessments of memory.
Methods: In a prospective, naturalistic study conducted at 7 hospitals in the NYC metropolitan area, 347 patients were evaluated by research assessors before, immediately after (mean=4 days), and 6 months after an index course of ECT. Evaluations included the Cognitive Failures Questionnaire (CFQ), a structured instrument to assess subjective cognitive complaints, and a novel global evaluation of anticipated effects on memory (prior to ECT) and of the actual effects on memory (postECT and 6-month follow-up). Depressive symptoms were assessed with the Hamilton Rating Scale for Depression (HRSD), and the primary neuropsychological measure was the Columbia University Autobiographical Memory Interview-Short Form (AMI-SF), which assessed retrograde amnesia.
Results: CFQ total scores markedly improved from preECT to postECT, paired-t (276)=4.48, $P<0.0001$. At 6-month follow-up, these scores were unchanged relative to postECT, paired-t (176)=1.01, P=0.32. Of 277 patients, 68.6% had fewer, 28.5% had more, and 2.9% had no change in cognitive complaints at postECT relative to preECT. At 6 months (N=167), these percentages were 66.5%, 29.3%, and 4.2%, respectively. Regression analyses were conducted predicting the postECT or 6-month CFQ scores with the baseline CFQ score, age, sex, premorbid IQ, postECT or 6-month HRSD score, change in AMI-SF scores, electrical waveform, and number of bifrontal, bilateral or right unilateral treatments as predictors. In both analyses the baseline CFQ and contemporaneous HRSD scores were robust predictors (all $P<0.0001$), with depression severity inversely related to cognitive complaints. Patients who received sine wave stimulation had more complaints at both time points than patients treated with brief pulse stimulation (all $P<0.01$). There were no associations with the objective measure of retrograde amnesia. These same patterns pertained to the CFQ memory subscale. In contrast, of 318 patients providing global ratings postECT, 51.9% indicated that they experienced a deleterious effect on memory, 27.7% stated there was no change, and 20.4% stated they experienced improvement. At 6-month follow-up (N=238), these percentages were 63.4%, 22.3%, and

14.3%, indicating more negative evaluations. Similar regression analyses were conducted on the postECT and 6-month global evaluations. At both time points, greater retrograde amnesia on the AMI-SF was associated with self-reports of more deleterious effects on memory (all P<0.05). At 6-month follow-up, a larger number of bilateral ECT treatments was also associated with more negative reports (P<0.02). Age and premorbid IQ showed especially strong associations at both time points (all P<0.0001), whereas HRSD scores were related to the global evaluations only at the postECT time point (P=0.0002).
Discussion: Structured assessments of cognition and direct questioning of global effects yield radically different assessments of the effects of ECT on memory. The former technique is strongly influenced by clinical state, whereas the global report appears to be more strongly associated with objective memory deficit, aspects of treatment technique, and demographic factors. This study provides the first evidence of covariation between self-evaluations and objective memory impairment.

57. Pharmacological Dissection of the Effect of Risperidone on SSRI-Induced Changes of Norepinephrine Neuronal Firing
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Background: Atypical antipsychotics have been shown to be effective agents in depressed patients not responding to selective serotonin (5-HT) reuptake inhibitors (SSRIs). Since the only properties that the atypical antipsychotics share is their capacity to antagonize dopamine type 2 (D2) and 5-HT2A receptors, and the addition of the D2 antagonist haloperidol to antidepressants is not helpful in depressed patients without psychotic symptoms, they may thus act via 5-HT2A receptor blockade. SSRIs attenuate locus coeruleus norepinephrine (NE) neuronal firing whereas the combination of selective 5-HT reuptake inhibition and 5-HT2A receptor blockade enhances NE release. Atypical antipsychotics have been shown to be effective agents in depressed patients not responding to selective serotonin (5-HT) reuptake inhibitors (SSRIs). Since the only properties that the atypical antipsychotics share is their capacity to antagonize dopamine type 2 (D2) and 5-HT2A receptors, and the addition of the D2 antagonist haloperidol to antidepressants is not helpful in depressed patients without psychotic symptoms, they may thus act via 5-HT2A receptor blockade. SSRIs attenuate locus coeruleus norepinephrine (NE) neuronal firing whereas the combination of selective 5-HT reuptake inhibition and 5-HT2A receptor blockade enhances NE release.
Methods: The effects of the atypical antipsychotic risperidone (1 mg/kg/day) and of the following antagonists: MDL 100,907 (5-HT2A; 0.5 mg/kg/day), SB 242084 (5-HT2C; 0.5 mg/kg/day), haloperidol (D2; 0.1 mg/kg/day) on NE neuron firing were thus examined alone (all given s.c. for 2 days in a single injection with a third dose injected immediately before the experiment) and in combination with escitalopram (10 mg/kg/day X 2 days administered using osmotic minipumps implanted s.c.) in chloral hydrate anesthetized rats.
Results: Escitalopram produced a 70% decrease in firing on NE neurons, whereas risperidone, MDL 100,907, SB 242084, and haloperidol were without effect on their own. The combination of SB 242084 and haloperidol did not modify the inhibitory action of escitalopram. In contrast, MDL 100,907 prevented the effect of escitalopram whereas risperidone plus escitalopram doubled the firing rate of NE neurons observed in the control rats.
Discussion: In conclusion, the SSRI-induced decrease in firing of NE neurons appears to be mediated by the activation of 5-HT2A receptors. Risperidone when combined with a SSRI exerts a potentiating action on the firing of NE neuronal firing. The basis for this unexpected effect of risperidone on NE neuronal function remains to be established. The prompt increase in NE function observed with this atypical antipsychotic medication, occurring only in the presence of 5-HT reuptake blockade, could explain, at least in part, the robust and rapid antidepressant response often obtained with this combination strategy.

58. Activity of a Selective Galanin-3 Receptor (GAL_3) Antagonist In Acute and Chronic Behavioral Models of Anxiety and Depression
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Background: Galanin is a peptide neurotransmitter that has been implicated in many physiological processes including feeding behavior, pain and depression (Bartfai et al., 1993; Kask et al., 1997; Weiss et al., 1998). The actions of galanin are mediated via three known G-protein coupled receptor subtypes: GAL_1, GAL_2 and GAL_3 (Branchek et al., 2000). Although GAL_1 and GAL_2 are widely distributed in the brain (O'Donnell et al., 1999), GAL_3 mRNA is restricted and is localized in areas such as the amygdala, locus coeruleus and dorsal raphe nucleus (Menniken et al., 2002). This distribution suggests a possible role of GAL_3 in limbic function. However, progress in determining a clear functional role of this receptor *in vivo* has been hampered by the absence of suitable ligands (Lu et al., 2005a).
Methods: N/A
Results: SNAP 37889 is a small molecule, high affinity, selective GAL_3 antagonist (K_i GAL_3=17nM; K_i $GAL_{1,2}$> 5000 nM). This compound displayed significant activity in animal models of anxiety and depression. In the acute stress-induced hyperthermia test, administration of SNAP 37889 (0.3-30 mg/kg, p.o.) to mice produced a significant, dose-dependent inhibition of the stimulated increase in core body temperature equivalent to that of the benzodiazepine positive control, chlordiazepoxide (CDP; 5mg/kg, p.o.). Likewise, SNAP 37889 was shown to significantly and dose-dependently increase punished drinking in the rat Vogel Conflict test (3, 30 mg/kg, i.p.) and to increase interaction time in the rat social interaction test (3-30 mg/kg, p.o.) similar to CDP following acute administration. The increase in social interaction was not accompanied by an increase in rearing behavior, suggesting that the effects of SNAP 37889 in this test were not due to a non-specific increase in locomotor activity. Acute anxiolytic-like activity was also noted in the guinea pig vocalization test, where this compound (3-30 mg/kg, p.o.) reduced the number of vocalizations emitted by guinea pig pups following maternal separation to a similar degree as the reference anxiolytic agent buspirone (2 mg/kg, p.o.). SNAP 37889 dose-dependently decreased immobility and increased swimming time in the acute rat forced swim test (1-10 mg/kg, p.o.), similar to the antidepressant fluoxetine (10mg/kg, p.o.). In chronic studies, SNAP 37889 increased social interaction time following 14-day treatment (30 mg/kg, i.p.), in contrast to repeated CDP administration (5 mg/kg, i.p.). Treatment with SNAP 37889 (30 mg/kg, i.p.) for an additional 7 days resulted in decreased immobility and increased swim time, indicating chronic anxiolytic- and antidepressant-like effects of GAL_3 antagonists.
Discussion: Galanin has recently been implicated in human depression (Murck et al., 2004) and gene expression analysis after antidepressant treatment in rats has focused attention on the potential role of GAL_2 receptor agonists in depression (Lu et al., 2005b). The present preclinical results suggest a role for the GAL_3 receptor in depression and anxiety and indicate that antagonists targeted to GAL_3 may serve as potential therapeutic agents for the treatment of human affective disorders.

59. Attention Bias to Threat in Bipolar Children With or Without Comorbid Anxiety Disorders
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Sponsor: Monique Ernst

Background: Anxiety disorders are common in both children and adults with bipolar disorder (BPD) (Dickstein et al, in press). Comorbid anxiety disorders may constitute a genetic subtype of BPD in

adults; however, this has yet to be explored in pediatric BPD. The identification of pathophysiological differences between BPD children with and without anxiety would have genetic and clinical implications. Disruptions in attention allocation to threat cues are seen in patients with anxiety disorders. Depending on methodological or clinical factors, threat cues can bias attention towards or away from a threatening stimulus. Using a visual-probe task, Pine et al (2005) demonstrated an attentional bias away from threatening faces in maltreated children. In adults with anxiety, however, there appears to be a bias toward threatening stimuli. These data indicate that threatening material disrupts attention allocation in patients with anxiety, but the nature of that disruption varies developmentally. We used the visual-probe task to ascertain the impact of a threat cue on attentional function in BPD children with and without an anxiety disorder (BPD+ANX vs. BPD-ANX). We hypothesized that BPD+ANX would demonstrate a bias away from threatening stimuli, as in children with anxiety disorders alone, whereas BPD-ANX would not differ from controls.

Methods: Pediatric BPD+ANX (N=21), BPD-ANX (N=11) and controls (N=18) were studied with the visual-probe paradigm. The task displays face-pairs, one with a neutral expression and one either angry (i.e., threatening) or happy (Mogg & Bradley, 1999). Trials include a 500 msec fixation cross followed by a face-pair for 500 msec, and then, a single-asterisk probe for 1100 msec on the left or right side. Subjects were instructed to press one of two keys as quickly and accurately as possible to indicate the probe location (right vs left side). There were two trial types of interest: (1) congruent trials: threat/neutral face-pair followed by a probe replacing the threat face; and (2) incongruent trials: threat/neutral face-pair followed by a probe replacing the neutral face.

Results: BPD+ANX (47.6% male; mean age = 14.1, SD 2.7), BPD-ANX (45.5% male; mean age = 14.4, SD 3.5) and controls (76.5% male, mean age = 14.0, SD 2.4) did not differ by age or gender. Attention bias was calculated by subtracting congruent from incongruent mean reaction time. Positive numbers indicate a bias toward anger/threat. As hypothesized, there was a relationship between anxiety and threat bias ($F = 3.3$, $p = .04$; BPD+ANX mean = 25.7, SD 28.1; BPD-ANX mean = .32, SD 31.9; control mean = 6.5, SD 29.1). However, unexpectedly, BPD+ANX demonstrated a stronger bias towards threatening faces than did BPD-ANX or controls.

Discussion: Studies have found that anxious children demonstrate a bias away from threat and anxious adults exhibit a bias toward threat. This was the first study to examine attention allocation to threat in BPD children. BPD+ANX demonstrated a significant bias toward threat, whereas children with anxiety disorders alone have a bias away from threat (Pine et al, 2005). Thus, anxiety disorders in the context of BPD may be "phenocopies" of anxiety disorders presenting alone; that is, different pathophysiological mechanisms may result in similar clinical presentations. Other studies (Dickstein et al, in press) have found that, compared with BPD-ANX, BPD+ANX have an earlier age of onset and more impairment, suggesting that comorbid anxiety in the context of pediatric BPD may represent a particularly severe phenotype of BPD. Future research should ascertain whether BPD+ANX is a distinct subtype of BPD.

60. Post-Retrieval Propranolol Weakens Longstanding Traumatic Memories

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Sponsor: Roger Pitman

Background: Administration of the β-adrenergic blocker propranolol following a psychologically traumatic event reduces the strength of its memory, measured by lower physiological responses during subsequent script-driven mental imagery.[1] Once the window of opportunity for influencing long-term memory consolidation has closed, β-blockers no longer would be able to exert this protective effect. Recent animal research suggests that this window may be reopened merely by reactivating the memory.[2] According to this view, retrieval returns the memory to a labile state, and it must then be reconsolidated to persist. In animals, propranolol reduces reconsolidation of inhibitory avoidance and auditory fear conditioning.[3] This process is neurophysiologically distinct from extinction. Reconsolidation blockade could lead to novel treatments for patients who have already developed post-traumatic stress disorder (PTSD)[4] in a manner parallel to the prevention of PTSD through consolidation blockade.[1] To date, the only report of reconsolidation and its blockade in humans involved a motor sequencing task that did not use drugs.[5] We tested whether reactivation of traumatic memories in PTSD patients followed by propranolol would weaken these disturbing and often disabling memories.

Methods: We used a validated psychophysiological script-driven imagery technique[6] to study 19 patients with chronic PTSD from various civilian traumatic events. Each patient described their traumatic event for approximately 10 minutes. Immediately thereafter, patients received either 40 mg short-acting propranolol followed an hour later by 60 mg long-acting propranolol (n=9), or placebo capsules (n=10) randomized double-blind. Staff then composed brief scripts portraying the event in the patient's own words and recorded them for playback in the laboratory. One week later, patients listened to their scripts and imagined the event as if it were happening again, while three physiological responses were measured. To reduce heteroskedasticity, responses were square-root transformed prior to analysis.

Results: Physiological responses during mental imagery of the traumatic event were significantly smaller in the PTSD patients who had received propranolol a week earlier. Placebo vs. propranolol mean responses (SDs) were: heart rate 2.0 (1.1) vs. 0.4 (2.2) $BPM^{0.5}$, $t(17)=2.0$, $p=0.03$; skin conductance 1.00 (0.55) vs. 0.37 (0.47) $\mu S^{0.5}$, $t(16)=2.6$, $p=0.01$; corrugator EMG 1.0 (1.7) vs. 0.6 (0.9) $\mu V^{0.5}$, $t(15)=0.6$, $p=0.28$ (univariate p's one-tailed); Hotelling's $T^2(3,16)=4.6$, $p=.01$.

Discussion: The findings are consistent with, but not demonstrative of, pharmacological blockade of the reconsolidation of traumatic memories. To confirm that reconsolidation is the operative mechanism, additional controls will need to be incorporated into future human studies, including a non-reactivated propranol group; measurement of post-reactivation, post-propranolol, short-term memory; and follow-up testing for spontaneous recovery. Regardless of the underlying mechanism, the finding that a brief evocation of the memory of a traumatic event immediately followed by a single (combined short- and long-acting) dose of a commonly used, safe medication can reduce physiological responses associated with the traumatic memory measured a full week later opens the door to exciting therapeutic opportunities for PTSD and possibly other psychopathology.

References:1. Biol Psychiatry 2002;51:189-92; 2. Nature 2000;406(6797):722-6; 3. Neuroscience 2004;129:267-72; 4. J Neurosci. 1999;19:6623-8 5. Nature 2003;425(6958):616-20; 6. Arch Gen Psychiatry 1987;44:970-5.

61. Serotonin Transporter Polymorphism Association with Neuroticism and Depression: A Controversy Evaluated

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Background: The serotonin transporter is the target of commonly used antidepressants, SSRIs, and thus a good candidate gene for association studies of genetic risk factors for depression and related traits. 5-HTTLPR is a common functional insertion/deletion polymorphism in the promoter of this gene - the short S allele produces less mRNA than the long L allele. Given the high impact of environmen-

tal risk factors such as significant life events on risk for depression itself, using an endophenotype that is highly correlated with depression but not so much affected by life events is a useful approach to identify genetic risk factors for depression. Neuroticism and other personality measures of anxiety, e.g. harm avoidance, are trait markers for risk for depression. At least in women, 60% of the genetic variance of Neuroticism overlaps that of depression, and thus findings for Neuroticism are likely to be relevant for depression.
Methods: We (Sen et al., 2004; Sen et al. 2005) and others (Schinka et al. 2004) have recently evaluated the now > 30 publications that evaluated association between 5-HTTLPR and anxiety-related traits, finding that the effect size of the association is small (about 0.1 SD or ~1.5% of the variance), and highly significant ($p<0.00002$) only when studies using NEO-PI-based measures were used. Studies using the TCI/TPQ have a slightly larger effect size but the association was not significant due to larger variation. Munafo et al. (Molecular Psychiatry online) recently found the opposite effect of inventory, i.e. small but significant association restricted to TCI/TPQ but no significant association with the NEO-PI. Munafo concluded that different inclusion and exclusion criteria (removal of studies with psychopathology and with deviation from HWE) caused the reversal of the conclusion. In order to test that idea, we used the same criteria to repeat the meta-analysis.
Results: Using the same inclusion and exclusion criteria used by Munafo, we confirm our previous results of association between 5-HTTLPR and anxiety measured by the NEO but not if measured by the TCI/TPQ. The TPQ shows an even larger effect size, which is, however, not significant due to high variance. We reach this conclusion regardless of dominance assumed, different inclusion and exclusion criteria, and specific meta-analytic methodologies. The less quantitative nature of the TPQ (yes/no instead of 1-5 scale for answers) and fewer items may contribute to the high variance and thus difficulty in finding this association significant in spite of a trend in the same direction.
Discussion: We conclude that meta-analysis is a robust method to evaluate small effects of association, and that although the effect size is small, 5-HTTLPR is associated with anxiety-related personality traits, and that this association is not sensitive to differences in meta-analytic methodologies. In addition, Caspi et al. (2003) found this variant to be associated with major depression once significant life events have been taken into account, a finding that was recently replicated (Kendler 2005). In contrast, the same group (Willis-Owen, Biol. Psych. 2005) has performed the largest single association study to date, using the extremes selected from a sample of >100,000 individuals. In spite of a power of 100% to detect even 0.5% effect size, no association between 5-HTTLPR and Eysenck's N or major depression (measured by mailed questionnaire) was found. How can a p value of <0.00002 in favor of association in several meta-analyses be compatible with no finding of an association in such a large selected study? We will discuss the effect of highly selected samples in normally distributed traits which could explain this discrepancy.

62. A Pilot Controlled Trial of Bupropion vs. Es-Citalopram in Generalized Anxiety Disorder (GAD)

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Sponsor: Samuel Eiduson

Background: Little is known about the effect of buspropion in the treatment of anxiety, despite the fact other drugs used to treat depression have established efficacy in reducing anxiety. Clinicians generally stay away from buproion when treating anxious patients due to reported increases in anxiety during treatment initiation. This is suprising given that most antidepressants including tricyclics and SSRIs have similar activating effects during treatment initiation. In bupropion the activating effects may be related to the hemodynamic properties in older formulations which may be reduced in more recent formulations i.e. Welbutrin XL. Tolerability and a better side effect profile warrant the evaluation of Wellbutrin XL in the treatment of anxiety. Our objective was to evaluate whether Es-citalopram or Bupropion XL would have preferential efficacy in reducing specific symptoms of GAD. We hypothesized that subjects treated with es-citalopram would have significant decreases in symptoms of fear whereas subjects treated with bupropion would have significant increases in their ability to cope with fear (resilience).
Methods: This study utilized a randomized, double-blind, dose-controlled, parallel-group design. Thirty-two outpatients with a mean age of 36±2.56 years were randomized into one of two treatment groups: Es-citalopram (20mg/day) or Bupropion (300mg/day). The primary efficacy measures were: the Hamilton Anxiety Rating Scale, Hamilton Depression Rating Scale and Self Efficiency Scale. Mean HARS total scores, HDRS total scores, and SES index of resilience were analyzed at each post randomization visit and endpoint using SPSS-11. ANOVA was performed to determine differences between treatment groups at baseline. ACOVA was performed using baseline as a covariate on each of the outcome variables.
Results: The baseline scores on HARS/HDRS were 24.88±4.58/12.53±4.14 for es-citalopram-treated subjects and 23.08±6.60/12.69±3.71 for bupropion-treated subjects. The end point means on the primary dependent variable HARS were 11.06±6.87 for es-citalopram-treated subjects and 4.54±2.75 for bupropion-treated subjects, which demonstrates a significant decrease in anxiety for both treatment groups. ANCOVA demonstrated HARS total score ($F= 9.46$, $p<0.005$) and HDRS total score ($F=0.65$, $p<0.025$) were significantly improved in Bupropion-treated subjects compared to es-citalopram-treated subjects at endpoint. Subjects treated with Bupropion had significantly lower SES scores compared to subjects treated with es-citalopram
Discussion: Subjects in both groups showed a decrease in anxiety symptoms over the 12-week treatment period. Subjects treated with bupropion showed significant decreases in anxiety symptoms compared to those treated with es-citalopram. Bupropion treated subjects showed a significant increase in self-sufficiency. Both treatments were equally well tolerated. The results of this study suggest bupropion may have a strong anti-anxiety property that was overlooked due to initial activating effects in earlier rapid absorbing formulations. A reduction of anxiety has been observed in several previously published trials utilizing bupropion for the treatment of depression, social anxiety disorder, and panic disorder. However, these findings stayed under clinicians' radar due to the initial activating effects of earlier formulations. This study is limited because of the small sample size and lack of placebo control, however, we believe it provides important preliminary information about the tolerablity and efficacy of bupropion in the treatment of GAD. The efficacy and tolerability of bupropion in GAD and other anxiety disorders should be further explored in larger placebo controlled trials.

63. Serotonin Transporter Binding in Unmedicated Bipolar Disorder Subjects using [Carbon-11] DASB and Positron Emission Tomography

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Background: Abnormalities of the serotonergic system have been hypothesized to play a role in the pathophysiology of bipolar disorder based upon based on findings that post-synaptic serotonin1A receptor binding and neuroendocrine responses to serotonin releasing agents and receptor agonists are decreased in bipolar depression. The serotonin transporter (5-HTT) function also was directly assessed in a small sample of six unmedicated type I bipolar disorder subjects using PET and [C-11]McN5652 which had inadequate specific to

non-specific binding to assess transporter levels in the cerebral cortex. This study reported a 22% increase in thalamic binding was detected that did not reach statistical significance, and no difference in midbrain binding between the BD and controls samples. In addition, a single post-mortem study in an bipolar sample (n=6), four of whom were medicated at the time of death, reported a 35% reduction in the frontal cortical (BA9) 5-HTT binding. The more recently developed 5-HTT radioligand [C-11]DASB, possesses sufficient specific-to-nonspecific binding to permit assessment of 5-HTT binding in frontal cortical as well as subcortical and brainstem areas. Thus, the present study included a larger unmedicated bipolar depressed sample and [C-11]DASB PET to assess 5-HTT function in depressed bipolar subjects relative to controls.
Methods: Sixteen currently-depressed, unmedicated subjects (12 female; mean age±sd=35±8) meeting Diagnostic Statistical Manual-IV criteria for bipolar disorder and 17 healthy-controls (11 female; 31±9) were underwent [11C]DASB PET scanning. The main outcome parameters of this study were 5-HTT binding potential and delivery. These were derived using the modified reference tissue model(Ichise et al. 2003) using cerebellum as the reference region. The relationship between illness severity as rated using the Montgomery-Asberg Depression, Young Mania and Hamilton Anxiety rating scales also was assessed.
Results: Serotonin transporter binding potential was greater in anterior cingulate cortex, rostral to the genu of the corpus callosum (33.8%; $p<0.001$), thalamus (16.2%; $p=0.033$) and lower in pontine raphe nuclei (9.9%; $p=0.064$) in bipolar subjects relative to controls.
Discussion: Serotonin transporter density and/or affinity may be increased in anterior cingulate cortex and thalamus whereas pontine raphe 5-HTT function appears to be downregulated or desensitized in bipolar disorder subjects. These findings may be compatible with the results of Agren et al (1992) who showed that while whole brain serotonin uptake via the blood brain barrier was decreased, serotonin turnover was increased in the pregenual anterior cingulate cortex during depression. The pregenual ACC also has been shown to have increased glucose metabolism during stress and depression. The serotonergic projections of the pontine raphe have been implicated in the regulation of appetite, feeding, aggression, sexual activity and sleep, behaviors commonly disturbed in depression.

64. Depressive-Like Effects of the Kappa-Opioid Receptor Agonist Salvinorin A on Behavior and Neurochemistry in Rats

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Background: Elevations in the activity of the transcription factor CREB (cAMP response element binding protein) within the nucleus accumbens (NAc) produce depressive-like behaviors in rats. This effect appears to be due, at least in part, to increases in CREB-mediated expression of dynorphin, an endogenous agonist at kappa-opioid receptors, in brain regions including the NAc. Here we examined whether systemic administration of salvinorin A (SalvA), a plant product that is a potent and highly selective kappa-opioid agonist, would produce depressive-like effects in the forced swim test (FST) and intracranial self-stimulation (ICSS) test, which are behavioral models often used to study depression in rats. In parallel, we examined the effects of the same treatments on activity levels in an open field. Finally, we examined the effects of SalvA on extracellular concentrations of dopamine (DA) and serotonin (5HT) in the NAc using *in vivo* microdialysis in awake and freely moving rats.
Methods: We first extracted, isolated, and purified SalvA from *Salvia divinorum* plant leaves. We then examined the effects of SalvA on immobility behavior in the FST, on locomotor activity within an open field, and on ICSS thresholds across a range of drug doses. We then compared the effects of behaviorally active and inactive doses of SalvA on DA and 5HT function within the NAc using microdialysis and HPLC.
Results: SalvA dose-dependently increased immobility in the FST, an effect opposite to that of standard antidepressant drugs. Doses that produced these effects in the FST did not affect locomotor activity in an open field. Furthermore, SalvA dose-dependently elevated ICSS thresholds, an effect similar to that produced by treatments which cause depressive symptoms in humans (e.g., drug withdrawal, antimanic agents). At a dose that caused the depressive-like effects in both the FST and ICSS assays, SalvA decreased extracellular concentrations of DA within the NAc without affecting extracellular concentrations of 5HT.
Discussion: These data provide additional support for the hypothesis that stimulation of brain kappa-opioid receptors triggers depressive-like signs in rats, and raise the possibility that decreases in extracellular concentrations of DA within the NAc contribute to these effects. These studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as promulgated by the National Institutes of Health. Supported by MH63266 (to WC) and the Stanley Medical Research Institute (to BMC).

65. Prefrontal Neurometabolite Changes Following Lamotrigine Treatment in Adolescents with Bipolar Depression

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Sponsor: Allan Reiss

Background: Lamotrigine has been found to be effective in treating depression in adolescents and adults with bipolar disorder (BD). Manic and depressive episodes may be associated with supranormal firing or increased excitatory neurotransmission and increased glutamate release, eventually resulting in local neurodegeneration. By modulating various ion channels, lamotrigine inhibits rapid firing of action potentials and excessive glutamate release, which could prevent neuronal toxicity and promote neuronal regeneration in relevant brain areas. Decreased metabolism, blood flow, and neuronal density in left dorsolateral prefrontal cortex (DLPFC) has been associated with unipolar and bipolar depression. Decreased levels of N-acetylaspartate (NAA), a marker of neuronal density, have been reported in DLPFC in patients with BD and MDD. However, it is not clear whether decreased prefrontal NAA reflects a static trait or changes with mood state in individuals with BD. NAA levels could possibly change after symptom resolution. Therefore, we hypothesized that left DLPFC NAA levels would increase in adolescents with bipolar depression after treatment with lamotrigine.
Methods: Eleven adolescents with BD I, II, or NOS, and experiencing a depressive episode, were enrolled in an 8-week open label trial of lamotrigine mono- or adjunct therapy. Response to treatment was defined by a week 8 score of 1 (very much improved) or 2 (much improved) on the CGI-I. Subjects were scanned with 1H-MRS at 3T, with a 8 cm3 voxel prescribed in the left DLPFC. Change in NAA/Cr ratios was the primary outcome measure and exploratory analyses of myo-inositol and choline ratios were also conducted.
Results: Mean age of subjects was 16.1 +/- 1.6 yrs. Four (36.4%) had a diagnosis of BD I, 3 (27.3%) BD II, and 4 (36.4%) BD NOS. Seven subjects (63.6%) were female. Ten subjects (91%) were considered responders. Final dose of lamotrigine was 136 +/- 28 mg/day. Three subjects (27.3%) were taking other psychotropic medications, with 1 subject taking aripiprazole, 1 taking valproate, and 1 taking methylphenidate. Wilcoxon signed ranked tests revealed a significant increase in left DLPFC NAA/Cr levels after 8 weeks of lamotrigine treatment (1.59 +/- .13 to 1.66 +/- .10, $p=.037$). There were no significant differences between baseline and follow-up Cr values (29.5 +/- 5.8 and 29.3 +/- 8.9, respectively). A significant increase was also detected in left DLPFC mI/Cr ratios (.49 +/- .06 to .56 +/- .11, $p=.038$).

Discussion: We found increases in left DLPFC NAA/Cr levels in adolescents with bipolar depression after 8 weeks of lamotrigine treatment. This is the first report of such neurometabolite changes in humans in response to lamotrigine. It is unclear whether lamotrigine had a direct effect in raising DLPFC NAA levels, a neurogenic effect leading to mood improvement, or if lamotrigine caused mood improvement in another way, which then led to a normalization of NAA levels, a neuroprotective effect. We also found significant post-treatment increases in mI/Cr levels. Previous research suggests that prefrontal mI concentrations are decreased compared to healthy controls in patients with BD during depressive episodes, whereas mI is increased during mania. Thus, our subjects may have experienced a normalization of mI levels in conjunction with moving from a depressed to euthymic mood state. The main limitation of this study was a small sample size, use of adjunctive medications, and use of ratios rather than absolute concentrations. Nonetheless, this study supports previous findings in animal research studies suggesting that lamotrigine has neuroprotective qualities. Further controlled studies with larger sample sizes are required to substantiate these findings.

66. The ERK Pathway in Left Anterior Cingulate Cortex Modulates Locomotive and Hedonic Activities

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Background: The extracellular signal regulated protein kinase (ERK) pathway is one of the intracellular signaling systems involved in neuronal development, survival, and long-term synaptic plasticity. Our previous studies reveal that treatment of rats or mice with antimanic mood-stabilizers, lithium and valproate, in clinical relevant manners activate the ERK pathway in prefrontal cortex and hippocampus. In addition, lithium and valproate promote the molecular and cellular function of ERK pathway such as BDNF and Bcl-2 expressions, neurogenesis, and neuronal survival. In animal behavioral studies we find that chemical inhibition of brain ERK pathway in rats reduces immobility in the forced swim test and increased locomotive/explorative activity in the large open field test. Our studies also show that ERK1 (one of two ERK subtypes) knockout mice are with brain regional specific functional deficit of the ERK pathway and exhibit reduced immobility in the forced swim test, increased activity in the open field test, persistently increased home-cage wheel running activity for at least 30 days, and enhanced response to psychostimulant.
Methods: In present study we examined the role of the ERK pathway as a behavioral modulator in left anterior cingulate cortex, one of the brain regions being implicated in pathophysiology of mood-disorders by human brain imaging and postmortem studies. In vivo gene delivery, brain stereotaxic surgery, chronic brain regional infusion, and behavioral tests were performed.
Results: Rats chronically infused with an ERK pathway inhibitor directly to left anterior cingulate cortex showed significant reduction of immobility in the forced swim test, increase in locomotive activities in the open field test, and enhancement of locomotive response to amphetamine. To further verify these findings, we developed a method to regionally express dominated negative ERK1 (to inhibit function of endogenous ERK) in left anterior cingulate cortex by injection of lentiviral vectors. Compared to the controls, rats injected with dominate negative ERK1 expression vector showed a trend of reduced immobility in the forced swim test, significant increases in activity in the open field test, significant increases in numbers of arm entries (without changing overall time spend in either open or closed arms) in the elevated plus maze test, and a significantly higher response to amphetamine. These rats also consumed more sweetened water in the sucrose and saccharin preference tests compared to control rats.
Discussion: Together, the body of data supports the role of the anterior cingulate in modulation of behaviors relevant to mood disorders; furthermore, the ERK pathway in the left anterior cingulate cortex is one of intracellular loops of neuronal circuitry that mediates hedonic and locomotive/explorative activities. The brain regional specific roles of the ERK pathway in the pathophysiology of mood disorders and in the therapeutic actions of antimanic agents are worthy for the further investigations.

67. Glucocorticoid Modulation of TPH2 Protein in Raphe Nuclei and Frontal Cortical 5-HTP Levels in C57/Bl6 Mice

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Sponsor: Ariel Deutch

Background: Dysregulation of the serotonergic system has been implicated in the pathophysiology of mood disorders (Lucki 1998; Owens 2004). Accordingly, there has been considerable impetus to understand the regulatory control of serotonin synthesis. Tryptophan hydroxylase (TPH) is the rate-limiting step in serotonin synthesis. Recent attention has focused on transcriptional regulation of the enzyme, including the ability of glucocorticoids to regulate TPH protein levels and activity in the rat (Azmitia and McEwen, 1969; Rastogi and Singhal, 1978; Azmitia and McEwen, 1974; Azmitia et al 1993; Singh et al 1992). However, these studies have not identified which of the two isoforms of TPH, TPH1 or TPH2, are regulated by glucocorticoids. We have examined this issue in the mouse. We recently found that daily treatment of C57/Bl6 mice with a glucocorticoid for four days caused a marked decrease (23% at 0.1 mg/kg to 44% at 3 mg/kg dexamethasone) in TPH2 mRNA levels, as detected by real-time RT-PCR, whereas TPH1 mRNA was unaffected (Clark et al., 2005). The glucocorticoid-elicited inhibition of TPH2 gene expression was blocked by coadministration of the glucocorticoid receptor antagonist mifepristone (30 mg/kg; RU-486).
Methods: TPH2-specific polyclonal antisera were generated to TPH-2 amino terminal peptides and validated using TPH-2 expressing tissues, cell lines and TPH2 transfected cells. TPH2 protein levels were evaluated using the TPH-2 antisera in Western blot analyses of mouse raphe homogenates from animals dosed with vehicle or dexamethasone once daily for four days. In vivo serotonin synthesis was determined by assessing the accumulation of 5-hydroxytrytophan (5-HTP) following inhibition of L-aromatic amino acid decarboxylase with NSD 1015. Clarified homogenates were analyzed using HPLC with electrochemical detection. Animal studies were carried out in accordance with the Merck IACUC Guidelines and the Guide for Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health.
Results: In order to further study regulation of TPH2 at the protein level, we generated TPH2-specific polyclonal antibodies, and used these antibodies to examine TPH2 expression in rodent and human raphe and pineal tissues, using both immunocytochemistry and Western blotting methods. We found that TPH2 protein levels in pontine raphe were dose-dependently decreased following four days of glucocorticoid treatment. Moreover, the glucocorticoid-elicited inhibition of TPH2 was functionally significant: serotonin synthesis was significantly reduced in the frontal cortex of glucocorticoid-treated mice, an effect that was blocked by mifepristone coadministration.
Discussion: Recently published genetic data from SNP analyses (Breidenthal et al 2004; De Luca et al 2004; Harvey et al 2004; Zill et al 2004) suggest that changes in expression and/or function of TPH2 may be associated with psychiatric disease and emphasize the need to understand both the regulation and function of the two TPH isoforms. We have shown that glucocorticoids can significantly affect serotonin biosynthesis through inhibition of TPH2 transcrip-

tion, suggesting that elevated glucocorticoid levels may be relevant to the etiology of affective disorders. Supported by Merck & Co., Inc.

68. Ontogeny of Individual Differences in Emotional Reactivity

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Sponsor: Huda Akil

Background: Outbred Sprague-Dawley rats can be classified as High Responders (HR) or Low Responders (LR) based on their levels of exploratory locomotion in a novel environment. While this novelty-seeking dimension was originally associated with differential vulnerability to substance abuse, findings on neuroendocrine responses to stress, anxiety-like behaviors, and neural gene expression suggest a fundamental difference in emotional reactivity between HR and LR animals. We recently initiated a selective breeding paradigm to enrich for HR and LR behavioral traits, in part, to track the developmental emergence of HR and LR phenotypes. After eight generations, selected lines show marked differences in responses to novelty as well as anxiety-like behavior. The major goals of the present study are to (1) examine the developmental emergence of the HR-LR phenotypes, both at the level of gene expression and behavior, and (2) evaluate whether environmental variables, such as maternal care, influence HR-LR differences in emotional reactivity.
Methods: Experiment 1: We first sought to investigate whether HR-LR behavioral differences are present in early life, representing lifelong traits, or whether these differences only emerge at certain critical periods of development (e.g. puberty). On postnatal day 25, Bred HR and LR rats were tested in either the Open-Field Test or Light-Dark Box. Brains from HR- and LR-bred pups were also dissected at several key developmental timepoints (postnatal days 7, 14, and 21), and microarray studies are currently underway to examine possible gene expression differences between the two Selectively Bred lines. Experiment 2: We previously observed behavioral differences between HR and LR mothers, so we hypothesized that these different mothering styles may influence the development of the HR-LR phenotype in their offspring. To examine the impact of maternal behavior, we applied a cross-fostering paradigm to our HR-bred and LR-bred lines. HR and LR litters were either raised by their biological mother, an HR- or an LR-foster mother. Adult offspring were screened for novelty induced locomotor activity, and then tested on the Elevated Plus Maze and Light-Dark Box anxiety tests.
Results: Experiment 1: Bred HR pups, like adult HRs, showed significantly greater locomotor activity, decreased anxiety-like behavior, and an exaggerated stress-evoked corticosterone response, compared to Bred LR animals. Experiment 2: Surprisingly, cross-fostering had no detectable impact on the locomotor response to novelty, although it did subtly influence anxiety behavior in HR and LR offspring.
Discussion: Results from these studies show that (1) phenotypic differences between HR and LR pups are already established in early life, prior to the onset of puberty. (2) The HR-LR phenotypes have a strong genetic component, although environmental factors such as maternal behavior, and possibly chronic stress during adolescence, exert some influence on the development of anxiety-like behavior, particularly in LR offspring. Ongoing studies will assess whether cross-fostering or other early life manipulation influence the stress responsiveness of HR-LR offspring or key aspects of their neuronal phenotype. Ultimately this model may help to dissect the complex interactions between genetic background and environmental changes during development, which result in differences in emotional reactivity in later life. Supported by N00014-02-1-0879, NIH P01 MH42251, and RO1 DA13386.

69. A Pilot Study of the Effect of Omega-3 Fatty Acids on Heart Rate Variability in Anxiety Disorders

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Sponsor: Jonathan Davidson

Background: There is evidence of an effect for omega-3 fatty acids (O3FA) in major depression and for comparable mood stabilizing properties in bipolar disorder. Recognizing the substantial overlap between symptoms of depression and anxiety and high rates of comorbidity between these disorders, it is possible that O3FA may have a role in treating anxiety. A potential role for O3FA in anxiety is suggested by animal data. One mechanism for the activity of O3FA may be through modulation of autonomic balance, as evidenced by improvement in heart rate variability (HRV), which has been found to be impaired in anxiety. Treatment with O3FA can increase HRV in survivors of myocardial infarction. The purpose of this study was to 1) collect pilot data on the effect of O3FA in patients with clinically significant anxiety disorders; and 2) to evaluate the effect of O3FA on heart rate variability in this population.
Methods: Adult outpatients age 18-60 with a primary DSM-IV anxiety disorder and a clinically significant level of symptoms were entered into the trial. Subjects with a history of cardiovascular disease, hypertension, or diabetes and those taking psychotropic medications or O3FA supplements were excluded. Eligible subjects received 8 weeks of open label treatment with an O3FA supplement (2100 mg EPA; 1500 mg DHA) daily. Baseline and post treatment assessments included measures of anxiety, depression, resilience, general psychopathology, global improvement, heart rate variability, and side effects. Treatment response was evaluated by the pre to post-treatment change in the Hospital Anxiety and Depression Scale (HADS) anxiety subscale score and was analyzed using a Wilcoxon Signed Rank Test. The effect of treatment on autonomic indices (HRV, baroreflex sensitivity, blood pressure, heart rate) and on affective variables of interest were compared using repeated measures ANOVA. Pre to post treatment changes in other measures were evaluated as secondary outcomes.
Results: 25 subjects were enrolled, 24 of whom returned for at least one post-baseline assessment. Subjects were predominantly females with either generalized anxiety disorder or social anxiety disorder. Significant improvement was observed on measures all clinical and self ratings ($p < .05$). However, no changes were found on autonomic indices. The treatment was well tolerated.
Discussion: Adults treated with O3FA supplements for 8 weeks demonstrate significant improvement in anxiety, depressive symptoms, general psychopathology, and stress coping. The mechanism by which this occurs does not appear to be through improvement in autonomic tone. Nonetheless, further controlled trials of fatty supplements in anxiety disorders are needed.

70. Adjunctive Eszopiclone and Fluoxetine in Major Depressive Disorder and Insomnia: Effects on Sleep and Depression

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Background: Insomnia can co-exist with depression. This study evaluated the efficacy of eszopiclone in patients with major-depressive-disorder (MDD) and co-morbid insomnia during concurrent fluoxetine treatment.
Methods: Patients who met DSM-IV criteria for MDD and insomnia received 10 weeks of fluoxetine QAM and were randomized to nightly eszopiclone 3mg (n=270) or placebo (n=275) for 8 weeks; additional inclusion criteria were sleep latency (SL) ≥30 minutes, wake-time-after-sleep-onset (WASO) ≥45 min, and total sleep time (TST) ≤390

min. Subjective sleep and daytime function were assessed weekly. Depression was assessed with the HAM-D17 (every 4 weeks) and the Clinical-Global-Impression Improvement (CGI-I) and Severity scales (CGI-S) each visit. Depression response=50% or greater decrease from baseline HAM-D17; remission=HAM-D17≤7.
Results: Compared with fluoxetine-placebo, fluoxetine-eszopiclone co-administration resulted in significantly decreased SL and WASO, and greater TST at each treatment visit ($p<0.04$); higher ratings across the treatment period in sleep quality and depth ($p<0.001$); and higher ratings of daytime alertness, ability to concentrate, and well-being ($p<0.004$). The Insomnia-Severity-Index indicated that more eszopiclone patients had no clinically meaningful insomnia at Week 8 (55% versus 37%, p=0.0004). Eszopiclone co-administration resulted in significantly decreased HAM-D17 scores at Week 4 (p=0.01) with progressive improvement at Week 8 (p=0.002). These differences remained significant after removing the insomnia items at Week 8 (p=0.04), but not at Week 4 (p=0.16). At Week 8, significantly more eszopiclone patients were responders (59% vs 48%, p=0.009) and remitters (42% vs 33%, p=0.03). CGI-I and CGI-S scores were significantly improved with eszopiclone co-administration ($p<0.05$). Treatment was well-tolerated, with similar adverse event and dropout rates. Unpleasant taste was more common with eszopiclone.
Discussion: In this study, eszopiclone/fluoxetine co-administration was well tolerated and associated with significantly improved sleep and daytime function. Significant improvements in several of the antidepressant measurements were observed in the eszopiclone/fluoxetine arm as compared to the placebo/fluoxetine arm in patients with MDD and insomnia.

71. Engraftment of a Monoaminergic Cell Line within Affective Neural Circuitry

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Grafting specific cell types into the brain and spinal cord has shown promise for repopulating neural cells, reestablishing connectivity, and restoring endogenous (or providing new) neuroactive agents. This work has been done primarily for clinical use in neurologic diseases, such as Parkinson's disease, Huntington's disease, dementia, epilepsy, stroke, and traumatic brain injury. With our expanding knowledge of the pathoetiology of mental illness, neural transplantation as a research tool and as a potential therapeutic approach for intractable psychiatric diseases has become tenable. Embryonic stem (ES) cells can be cued to differentiate into specific phenotypes and therefore may have applications in the study, and perhaps the treatment, of neuropsychiatric disorders. The anterior cingulate cortex (ACCx) has been implicated in the pathoetiology of a variety of such neuropsychiatric disorders, including depression. The present experiments investigate the ability of ES cells with monoaminergic potential to engraft into this region of the corticolimbic system and exhibit antidepressant and anxiolytic effects.
Methods: This series of studies utilized an embryonic stem cell line (N2) transfected with Nurr1, a transcription factor important in the differentiation of midbrain cells into dopaminergic (DA) neurons. Cultured N2 cells indeed become dopaminergic, and under special culture conditions, a substantial population of cells can be cued to differentiate into serotonin (5HT)-immunoreactive neurons. Suspensions of N2 cells were micrografted bilaterally into rat medial prefrontal cortex (mPFC), the rodent homologue of human ACCx. Behavioral effects of the grafts were examined utilizing models of anxiety and models of depression. 5-HT and DA neuron survival and integration were evaluated using immunofluorescence, and as a surrogate for anti-depressant effects, graft-induced neurogenesis was also examined with bromo-deoxyuridine (Brdu).
Results: Status-post 6 to 12 weeks transplantation, grafts were found to contained large numbers of surviving 5HT- and TH/Dopamine Transporter (DAT)-positive neurons having robust neurite integration with the host. Furthermore, neurogenesis was increased in the dentate gyrus of transplanted animals. Behavioral testing revealed significant graft effects in models of both anxiety and depression.
Discussion: The present findings support the hypothesis that customized cell lines can be effectively engrafted into affective circuitry thereby modifying the functional anatomy of emotion. It is important to note that antidepressant medicines demonstrate efficacy in treating anxiety disorders as well as mood. N2 cells engrafted into the mPFC appear to integrate into this cortico-limbic site, perhaps supplementing levels of 5HT and DA, and possibly influencing distant brain regions, such as the amygdala and hippocampus, ultimately resulting in improved performance in animal models of affective disorders. Furthermore, increases in Brdu-labeled cells within the dentate gyrus is consistent with neurogenesis seen with standard therapies for depression. These results indicate that transplanted cells could conceivably potentiate both 5HT and DA in a similar manner as antidepressant medicines. However, these engrafted neurons may have the advantage of providing permanent, ongoing, and perhaps more stable levels of monoamine activation. Moreover, if integrated into the corticolimbic network, transplanted neurons may be subject to modulation imposed by the circuitry within which they have integrated.

72. Cardiovascular Effects of Duloxetine: Preclinical and Clinical Findings

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Sponsor: Smriti Iyengar

Background: The tricyclic antidepressants are known to have adverse effects on several cardiovascular parameters. In contrast, selective serotonin reuptake inhibitors (SSRIs) incur minimal cardiovascular risk aside from sporadic cases of bradycardia with a recent study suggesting SSRIs may reduce cardiac morbidity and mortality in patients with a recent acute myocardial infarction (Taylor et al., 2005). Duloxetine is a recently approved serotonin norepinephrine reuptake inhibitor (SNRI) for both major depressive disorder (MDD) and diabetic peripheral neuropathic pain (DPNP). This review summarizes the cardiac effects of duloxetine based on results from in vitro and animal studies, and human trials including higher doses and chronic treatment.
Methods: The affinity of duloxetine for cardiac ion channels such as hERG, which predicts prolongation of the QT interval, was determined in vitro in a stably expressed human cell line. Cardiovascular parameters were also evaluated in single- and repeat-dose studies in rats and/or dogs. In humans, cardiovascular safety was analyzed (1) in healthy volunteers receiving duloxetine doses up to 400 mg/d to assess safety including cardiac effects (Derby et al., 2005; Zhang et al., 2005); (2) in 8 placebo-controlled MDD clinical trials, with paroxetine as an active comparator in 4 trials and fluoxetine in 2 trials where therapeutic doses of duloxetine ranged from 40 -120 mg/d for 8 or 9 weeks (Thase et al, 2005); (3) in a 52 week open-label long-term safety study of duloxetine (80-120 mg/d) (Raskin et al., 2003) and; (4) in a 12-week study of duloxetine versus venlafaxine in MDD (Perahia et al., 2005).
Results: The in vitro study found that duloxetine, at the maximum unbound plasma concentration observed clinically, had no adverse effect on any of the human cardiac ion channels tested, including hERG. In vivo, blood pressure (BP) and heart rate were not significantly altered in conscious animals following single oral doses of 7 or 20 mg/kg in rats and 10 mg/kg in dogs. Cardiac rhythm, conduction, and heart rate were unaffected in dogs by up to 1 year of treatment

with 3, 10 or 30 mg/kg. In healthy volunteers, duloxetine (even at 400 mg daily) had only a modest effect on elevation of BP and heart rate with no prolongation of the QTc (corrected QT) interval. In patients with MDD, small increases in heart rate and BP were observed. Although there were statistically significant differences in heart rate from baseline to endpoint between duloxetine versus fluoxetine or paroxetine, these changes were clinically insignificant. No significant differences in BP occurred between duloxetine and the aforementioned SSRIs (Thase et al., 2005). In the study of duloxetine (60-120 mg/day) and venlafaxine (75-225 mg/day) in MDD, no statistically significant differences occurred in elevated heart rate and BP except for significantly more venlafaxine patients with sustained systolic BP during the first phase (6 weeks) of the study.
Discussion: The preclinical data help explain the relatively low levels of cardiovascular adverse effects observed in humans. Consistent with the lack of effect on human cardiac ion channel binding in vitro, and with the lack of effect on conduction parameters in repeat-dose studies in dogs, no QTc prolongation was seen in humans. Consistent with the heart rate and BP findings in animals, there are relatively few small effects on these measures in clinical populations. In summary, duloxetine has a good cardiovascular safety profile, possibly due to lack of affinity for crucial cardiac ion channels and/or other unknown factors.

73. A Psychosocial Approach to Depression in Parkinson's Disease
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Sponsor: Javier Escobar

Background: Depression, the most common non-motor disturbance in Parkinson's disease (PD), is linked with a faster progression of physical symptoms, greater cognitive decline, and poorer quality of life. At present, medication treatment of depression is the standard approach despite the lack of definitive data, tolerability concerns, and sometimes, patient resistance. In non-PD populations, non-pharmacological approaches to depression (e.g., cognitive-behavioral therapy) are efficacious alternatives to antidepressants. There have been, however, no systematic attempts to evaluate these psychosocial techniques in individuals with PD. The present study was conducted to examine the feasibility and effectiveness of an individual cognitive-behavioral treatment package (based on A.T Beck, 1979) that was modified to meet the unique needs of the PD patient and was augmented by a caregiver focused social support intervention.
Methods: Ten patients with PD, meeting DSM-IV criteria for either Major Depressive Disorder or Dysthymia, participated in the study with a caregiver. Patients ranged in age from 52 to 76 with the duration of illness ranging from 1 to 9 years. All patients had a history of poor tolerability or lack of efficacy of antidepressant medication. The patients' 10-session study treatment involved behavioral activation (with an emphasis on exercise and pleasurable/meaningful activities), relaxation training, sleep hygiene and cognitive restructuring. Only one main topic was addressed in each session, with information streamlined, presented in a variety of formats (e.g., oral, written, audiotape), and frequently summarized. Caregivers attended 3-4 psychoeducational sessions, occurring separately from the patients' treatment sessions, which focused on coping skills and strategies for offering appropriate support while responding to the patients' negative thoughts in a constructive manner. The patients' depression (HAM-D, BDI), anxiety (SEQ), negative inferences (IQ), and perception of social support (AIFQ), and their caregivers' knowledge and provision of appropriate types of support (AIFQ2) were assessed at baseline, midpoint, post-treatment, and 1-month follow-up.
Results: The first five patients have finished the study to date, while the remaining five are in the process of completing the treatment program. Preliminary results have indicated that both patients and caregivers found the treatment package desirable, practical, and helpful. In particular, caregivers reported that the social support training decreased their feelings of helplessness and frustration by providing them with specific skills that could be applied on a daily basis, while patients experienced a significant reduction in depressive symptoms and negative cognitions, as well as an increased perception of social support. Complete data will be available by 11/05.
Discussion: To date, no comprehensive psychosocial treatment model exists for depression in PD. Individual cognitive-behavioral treatment, when modified appropriately, may be a feasible and effective option for PD depression. Larger, randomized controlled trials are needed to further evaluate the efficacy of this intervention and identify specific mechanisms of change.

74. Serotonin Attenuates Mating-Induced Glutamate Activity in the Medial Preoptic Area: Implications for Impaired Libido Resulting from SSRIs
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Sponsor: Travel Awardee, NIMH, 2005

Background: The medial preoptic area (MPOA) is essential for the regulation of male sexual behavior in all studied species, including humans. In support of this idea, studies show that ablations of the MPOA impair, whereas stimulation facilitates, male sexual behavior. Furthermore, mating increases neural activity in the MPOA of rodents, as evidenced by immunohistochemical and electrophysiological measures. The mechanisms responsible for activation of the MPOA are not entirely understood. We recently reported that mating evokes increased levels of glutamate in the MPOA. Moreover, reverse dialysis of glutamate uptake inhibitors enhanced both mating-induced release of glutamate in the MPOA and male sexual behavior, whereas microinjections of glutamate receptor antagonist impaired sexual behavior. These data indicate that glutamate may be responsible for mating-induced activation of the MPOA. Increased levels of serotonin (5-HT) inhibit sexual behavior. For example, men who take antidepressants of the selective serotonin reuptake inhibitor class (SSRIs) experienced impaired erection, ejaculation, and decreased libido. Similar observations were made in male rats given fluoxetine (Prozac). Here we examine whether increased 5-HT alters mating-induced release of glutamate in the MPOA and male sexual behavior.
Methods: We reverse-dialyzed 5-HT through concentric microdialysis probes placed in the MPOA of male rats; concurrently we collected 2-min samples for analysis of glutamate and measured sexual behavior. The microdialysis samples were later analyzed using high performance liquid chromatography with electrochemical detection.
Results: Analysis of microdialysis samples revealed that sexual activity increased levels of glutamate in the MPOA of male rats; glutamate levels peaked in the sample during which the male ejaculated and decreased precipitously during the post-ejaculatory refractory period. Moreover, reverse dialysis of 5-HT into the MPOA attenuated mating-induced increases in glutamate and also impaired male sexual behavior.
Discussion: These data suggest that one possible mechanism by which SSRIs impair male sexual behavior is by inhibiting mating-induced neural activity in the MPOA that results from release of glutamate.

75. Association of the Functional -1019C/G 5-HT1A Polymorphism with Decreased Prefrontal Cortex Activation Measured with 3T fMRI in Panic Disorder
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Sponsor: Godfrey Pearlson

Background: Panic disorder is an anxiety disorder characterized by sudden and unexpected panic attacks, anticipatory anxiety and a life-

time prevalence of 1-3% (Weissman et al., 1997). Family and twin studies propose that genetic factors contribute to the pathogenesis of the disorder with an estimated heritability of up to 48% (Hettema et al., 2001). Serotonergic genes (5-HTTLPR, 5-HT1A) and/or gene function have been implicated in the pathogenesis of panic disorder on the one hand (e.g. Lesch et al., 1996; Neumeister et al., 2004; Rothe et al., 2004) and amygdala function in response to fearful stimuli in healthy volunteers on the other hand (e.g. Hariri et al., 2002). Consequently, neuronal activation patterns following emotional stimulation in brain regions critical for emotional and learning processes might serve as a novel endophenotype for genetic studies in panic disorder.

Methods: In the present study, we therefore investigated regional brain activation in response to visual presentation of happy, fearful and angry face stimuli by means of fMRI at 3T in a sample of 20 patients with panic disorder (female=12, male=8). Voxel values of 5 x 2 predefined regions of interest (ROI) were extracted, summarized by mean and tested among the different conditions using the MarsBaR toolbox. Additionally, all patients were genotyped for the functional -1019C/G 5-HT1A and 5-HTTLPR polymorphisms according to published protocols (Rothe et al., 2004; Deckert et al., 1997). Genotype group differences in fMRI activation were analyzed using the Mann-Whitney-U-Test, where an influence of age, gender, marital status, medication and comorbid social phobia or depression was excluded.

Results: In patients homozygous for the 5-HT1A -1019G risk allele, fearful stimuli were associated with decreased activation in the right ventromedial prefrontal (p=0.01), right orbitofrontal cortex (p=0.04), and the right anterior cingulate cortex (p=0.03). In contrast, in response to happy faces patients homozygous for the 5-HT1A -1019G risk allele (p=0.03) and patients carrying the short risk allele of the 5-HTTLPR (p=0.05) showed significantly increased amygdala activation.

Discussion: Our data may indicate that the functional -1019C/G 5-HT1A variant has a significant influence on impaired cerebral processing of anxiety-related stimuli in patients with panic disorder. Processing in cortical regions known to play a crucial role in the evaluation of emotional stimuli and determining salient events is affected. Possibly, this is due to a differential prefrontal cortex serotonergic tonus in panic patients associated with a lack of inhibition of amygdala activation by the prefrontal cortex. Interestingly, however, in contrast to preceding imaging genomic studies on emotional processing and the serotonin system in healthy volunteers, in our panic disorder sample, we observed an influence of the serotonergic risk alleles on increased amygdala signalling in response to happy rather than threatening faces. In conclusion, our data provide preliminary evidence for an influence of the functional -1019C/G 5HT1A promoter polymorphism on prefrontal cortex activation patterns in response to anxiety-related stimuli in patients with panic disorder.

76. Membrane Localization of the G protein Gsα is Altered in Post-Mortem Brain of Depressed Suicide Victims: Possible Disruption of Plasma Membrane Rafts

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Sponsor: Mark Rasenick

Background: There has been much recent interest in the organization of G protein signaling complexes at the plasma membrane and the incorporation of these complexes into cholesterol and sphingolipid-rich membrane domains or rafts. Recent in vivo (rat) and in vitro (C6 rat glioma cells) studies have demonstrated that Gsα migrates from a Triton X-100 (TTX-100) insoluble membrane domain (TIMD or raft) to a TTX-100 soluble non-raft membrane domain in response to chronic treatment with tricyclic or SSRI antidepressants (Toki et al., 1999 J. Neurochem. 73: 1114-1120; Donati and Rasenick, 2005 Neuropsychopharm. 30: 1238-1245). Very closely related compounds, such as chlorpromazine (an antipsychotic) or an inactive fluoxetine analog (LY 368514) had no effect. Chronic antidepressant treatment is known to upregulate cAMP signaling, and recent evidence suggests that Gsα signals more effectively when outside of lipid raft fractions (Allen et al., 2005, Mol. Pharm. 67; 1493-1504).

Methods: Lipid raft Gsα content is determined with sucrose density gradients or with a sequential detergent extraction of a membrane sample with TTX-100 followed by TTX-114 (a stronger detergent than TTX-100).

Results: Using this method, Gsα was shown to migrate out of lipid raft domains and into a more facile association with adenylyl cyclase subsequent to chronic antidepressant treatment. Thus, it was suggested that since antidepressants moved Gsα out of raft domains, Gsα may move into those rafts in depressed humans. There is increasing evidence that there are alterations in the levels of proteins involved in the adenylyl cyclase signaling cascade in the brains of depressed suicide victims (Dwivedi et al., 2004 Biol. Psychiatry 55: 234-243; Pandey et al., 2005 Neuropsychopharm. Online publication). This study focuses on the localization of Gsα in the cerebellum and cortex of brains from both non-psychiatric control subjects and depressed suicide victims (brain tissue was obtained from the Brain Collection Program of the Maryland Psychiatric Research Center, Baltimore MD, in collaboration with the Medical Examiner's Office of the state of Maryland). Sequential TTX-100 and TTX-114 extractions reveal that there is 2 fold more TTX-100 soluble (non-raft) and 50% less TTX-114 soluble (TIMD or raft) Gsα in the cerebellum of control (n=10) vs. depressed suicide victims (n=10). The ratio of TTX-100/TTX-114 soluble Gsα is also nearly 2:1 for control vs. depressed suicide victims. Similar, though preliminary, results with cortex samples from each group (n=2) demonstrate a similar trend. The cortex studies are ongoing and updated data will be presented.

Discussion: These results suggest that depression is accompanied by movement of Gsα to a membrane domain where it is less likely to couple to adenylyl cyclase and that antidepressants may upregulate Gsα signaling via disruption of the lipid environment in which Gsα is normally ensconced.

77. Lithium Normalizes Dendritic Localization of Val66met Polymorphism of Brain-Derived Neurotrophic Factor (BDNFmet): Therapeutic Implications

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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: Mood disorders have traditionally been conceptualized as neurochemical disorders, but there is now evidence from a variety of sources demonstrating regional reductions in CNS volume as well as reductions in the numbers and/or sizes of glia and neurons in discrete brain areas. Although the precise cellular mechanisms underlying these morphometric changes remain to be fully elucidated, the data suggest that severe mood disorders are associated with impairments of structural plasticity and cellular resilience. Brain-derived neurotrophic factor (BDNF) plays an important role in promoting and modifying growth, development, and survival of neuronal populations, and, in the mature nervous system, is involved in activity-dependent neuronal plasticity. Based on several lines of evidence, BDNF has been hypothesized to play an important role in the pathogenesis of mood disorder, with two recent family-based association studies have provided evidence that a naturally occurring polymorphism in the pro-BDNF molecule shows an association with disease susceptibility in Bipolar Disorder. Notably, the valine (val) to methionine (met) substitution in the 5' pro-region of the human BDNF protein is associated with lower depolarization-induced secretion, and a failure to localize to secretory granules or synapses. Lithium has recently

been shown to exert neurotrophic effects in a variety of paradigms; thus, we sought to determine the role of lithium potentially correcting the aberrant BDNF dendritic localization and secretion conferred by the Val66Met pro-BDNF polymorphism.
Methods: Cortical neurons (8 DIV) were treated with lithium (1.0mM) for three days and then were infected with Sindbis virus constructs containing BDNF-met-GFP or BDNF-val-GFP. After 24-48 hours, images were acquired by confocal microscope and images were analyzed with 510-meta software.
Results: Consistent with previous results, we found that dentritic distribution of BDNF-met was significantly reduced compared to the BDNF-val. Lithium treatment significantly enhanced dendritic localization of BDNF-met form by ~40%, up to levels which were comparable to the BDNF-val form. The effect of lithium on BDNF-met secretion in response to depolarization is under investigation.
Discussion: These results suggest that lithium may have utility in correcting BDNF trafficking/secretion associated with genetic polymorphisms, and thus in the treatment of a variety of neuropsychiatric disorders.

78. Serotonin Transporter Activity Modulates 5-HT1B-Induced Alterations in Prepulse Inhibition and Locomotion: Implications for Obsessive Compulsive Disorder and Panic Disorder
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Sponsor: Rene Hen

Background: Serotonergic alterations have been implicated in both obsessive-compulsive disorder (OCD) and panic disorder (PD). Patients with OCD and PD exhibit reduced prepulse inhibition (PPI), which refers to the reduction of the startle response that occurs when a startling stimulus is preceded by a barely detectable prepulse. The mechanism underlying this abnormality has not been defined, but might involve abnormal signaling via 5-HT1B receptors. Acute challenge with 5-HT1B agonists exacerbates symptoms in OCD and PD patients. Moreover, 5-HT1B agonists reduce PPI and induce perseverative hyperlocomotion in rodents. Chronic administration of selective serotonin reuptake inhibitors (SSRIs) relieves symptoms of OCD and PD and desensitizes presynaptic 5-HT1A and 5-HT1B receptors. Several studies have linked the long allele (higher activity) variant of the serotonin transporter gene (5-HTT) promotor polymorphism with OCD or PD, although others have found no relationship. The mechanism by which variation in 5-HTT function might confer risk for OCD or PD is unknown. We evaluated potential mechanisms using preclinical models that assessed the effects of 5-HT1B agonists on PPI, an endophenotype for the sensorimotor gating deficits in OCD and PD, in mice with normal or reduced 5-HTT activity. We hypothesized that lower 5-HTT activity, induced by chronic SSRI or genetic knockout, would reduce the PPI deficits and perseverative hyperlocomotion induced by 5-HT1B agonists.
Methods: We first assessed the effects of acute injection with the 5-HT1B agonist RU24969 (0, 10 mg/kg), on PPI and locomotion in adult Balb/cJ mice receiving chronic (21 days) of fluoxetine (0, 10, 18, 25 mg/kg/day). We then assessed the effects of acute injection with RU24969 (0, 10 mg/kg) or the 5-HT1A agonist 8-OH-DPAT (0, 1 mg/kg) on PPI and locomotion in Balb/cJ mice receiving subchronic (4 days) or chronic of fluoxetine (0, 15 mg/kg/day). We then evaluated the effects of the same acute drug challenges on PPI and locomotion in 5-HTT wild-type (WT), heterozygous (HT), and knockout (KO) mice. Finally, Balb/cJ mice receiving subchronic or chronic fluoxetine were assessed for 8-OH-DPAT-induced hypothermia; 5-HTT WT, HT, and KO mice were also assessed.
Results: RU24969 disrupted PPI and induced hyperactivity in Balb/cJ mice receiving control, or subchronic fluoxetine, but not chronic fluoxetine. 8-OH-DPAT reduced locomotion and increased PPI regardless of fluoxetine treatment. Similarly, RU24969 disrupted PPI and induced hyperactivity in WT, but not 5-HTT KO mice. 5-HTT HT mice displayed intermediate phenotypes. 8-OH-DPAT altered locomotion in 5-HTT WT and HT, but not KO mice. Both subchronic and chronic fluoxetine treatment prevented 8-OH-DPAT-induced hypothermia; likewise, 5-HTT knockout blocked 8-OH-DPAT-induced hypothermia, and 5-HTT HT mice were intermediate.
Discussion: Reductions in 5-HTT activity, achieved via chronic SSRI treatment or genetic manipulation, results in profound desensitization of 5-HT1B receptors involved in the modulation of PPI and locomotion. 5-HT1A receptors involved in PPI or locomotion appear unaffected by chronic SSRI treatment or 5-HTT genetic manipulation, although those involved in temperature regulation were desensitized by both manipulations. High 5-HTT activity (long allele) may confer risk for OCD and PD by increasing sensitivity of 5-HT1B receptors, which disrupt PPI and induce perseverative motor behavior when activated. Autoradiographic studies assessing 5-HT1B binding and functional coupling are currently underway.

79. Underdiagnosis of Psychotic Symptoms in Patients with Bipolar Mania
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Background: Psychotic symptoms are often present in patients with bipolar mania, with reported rates as high as 68% (Pope & Lipinski, 1978; Coryell et al, 2001). The literature suggests that 48% of manic episodes present with at least 1 delusion, 15% with 1 hallucination, and 19% with 1 "thought disorder" (Goodwin & Jamison, 1990). The presence of psychosis may even be more common than is typically diagnosed, yet its recognition and treatment are important components in the effective management of these patients. The objective of the current analysis was to further explore this spectrum of psychosis in patients with bipolar mania.
Methods: Analysis of baseline data from 2 placebo-controlled studies of patients with an acute episode of bipolar mania with or without psychotic features. The spectrum of psychosis was defined as a score of mild (3) or greater on any of the following PANSS items: delusions, grandiosity, suspiciousness, hallucinations, conceptual disorganization, difficulty in abstract thinking, unusual thought content. Grandiosity, a hallmark feature of mania, was assessed for the presence of delusional content (PANSS score of ≥4).
Results: Data were available for 515 patients; 264 (51.3%) were diagnosed with psychotic features at study entry. Among all 515 patients, 94.6% had at least a mild rating on ≥1 of the psychosis items described above, 81.7% on ≥2 items, and 61.9% on ≥3 items. Even among the 251 diagnosed without psychotic features, 88.8% had at least a mild rating on ≥1 item, 63.7% on ≥2 items, and 38.2% on ≥3 items. Among all patients, 62% had grandiosity ratings of delusional proportion. Even among patients without a diagnosis of psychosis at study entry, 45% had such ratings.
Discussion: These preliminary findings are consistent with the literature and show high rates of psychosis in patients with bipolar mania. Further, a continuum of psychosis exists in these patients that may be under-recognized. For example, grandiosity is often rated as delusional even in patients without a formal diagnosis of psychotic features. Thus, these data suggest that careful patient evaluation is important to identify psychotic symptoms in patients with bipolar mania. Supported by Janssen L.P.

80. Olanzapine/Fluoxetine Combination Versus Lamotrigine in the Long-Term Treatment of Bipolar I Depression
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Background: To determine the efficacy and tolerability of olanzapine/fluoxetine combination (OFC) compared with lamotrigine (LMG) for the long-term treatment of bipolar depression.

Methods: This 25 week randomized, double-blind study compared OFC (6/25, 6/50, 12/25, or 12/50 mg/day, n=205) with LMG titrated to 200 mg/day (n=205) in patients with bipolar I disorder, depressed. Outcome measures included the Clinical Global Impression Severity (CGI-S) (primary), Montgomery-Asberg Depression Rating Scale (MADRS), and Young-Mania Rating Scale (YMRS) total scores.
Results: At baseline, patients were acutely depressed (mean MADRS=31.0) and had low mania scores (mean YMRS=4.9). Patients treated with OFC had significantly greater improvement than LMG-treated patients across the 25-week treatment period on CGI-Severity (p=.008), MADRS (p=.005), and YMRS (p<.001). Time to response (MADRS decrease of 50% or more) was significantly shorter for patients treated with OFC (21 vs. 33 days, p=.013). For those patients who were in remission (MADRS<=12) after the 7-week acute phase, the subsequent 18.2% (14/77) LMG vs. 13.7% (13/95) OFC relapse rate (MADRS>15) was not significantly different by treatment (p=.528). The rate of treatment-emergent mania was only 7.3% (14/191) vs. 5.0% (10/202) for LMG- vs. OFC-treated patients (p=.401). Adverse events in >10% of patients in either treatment group and more frequent (p<.05) in one group were somnolence, increased appetite, dry mouth, sedation, weight gain, and tremor in OFC-treated patients and insomnia in LMG-treated patients. Most adverse events developed within 3 weeks of treatment including 78% of the increased appetite cases in OFC-treated patients. There were 3 patients with suicide attempts and 5 with suicidal ideations in the LMG treatment group and 1 patient with a suicide attempt and 2 with suicidal ideation in the OFC treatment group. There was a significant difference in incidence of treatment-emergent cholesterol >=240: OFC 15.9% vs. LMG 3.7% (p<.001) and weight gain of >7%: OFC 33.8% vs. LMG 2.1% (p<.001).
Discussion: Patients with acute bipolar depression had significantly greater improvement over 25 weeks of treatment in both depressive and manic symptoms on OFC compared with LMG. There was no treatment difference in the incidence of relapse. Patients treated with OFC had more treatment emergent adverse events and a greater incidence of weight gain and treatment emergent high cholesterol.

81. A Comparison of ETRANK, MMRM and LOCF Analyses of Eighteen Placebo-Controlled Venlafaxine Clinical Trials for the Treatment of Major Depressive Disorder

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Sponsor: Michael Thase

Background: The inevitability of patient attrition from randomized controlled trials (RCTs) has led to the development of statistical methods to deal with missing data points. Mixed-model repeated measure (MMRM) and ETRANK analyses have been theorized to provide a more accurate assessment of outcomes than the traditional method of carrying the last observed data point forward (LOCF) for patients who drop out. As LOCF was the a priori method used in analysis of all phase II and III placebo-controlled RCTs of venlafaxine therapy of major depressive disorder (MDD), we were able to compare these newer methods against the older standard.
Methods: The present analysis was conducted using LOCF, MMRM and ETRANK (three different statistics) methods; change from baseline on the 17-item Hamilton Ratings Scale for Depression (HAM-D_{17}) was the dependent variable. Remission of symptoms, defined as HAM-$D_{17} \leq 7$, also was analyzed using LOCF_Logistic Regression and the Glimmix MMRM analysis.
Results: Two-thirds of the 18 LOCF analyses revealed significant drug-placebo differences. By contrast, 10 (56%) of the MMRM comparisons and 41 of 54 (76%) ETRANK comparisons demonstrated a significant difference. 42 (77.8%) ETRANK comparisons yielded a lower or equally significant difference compared to MMRM. 5 (27.8%) MMRM analyses had a lower or equally significant difference than ≥1 ETRANK. 5 (27.8%) LOCF comparisons had a lower significant difference than ≥1 ETRANK analysis. 12 (66.7%) LOCF comparisons had a lower significant difference than MMRM. Venlafaxine remission rates were found to be significantly different from placebo in 7 (38.9%) Glimmix analyses and 6 (33.3%) LOCF_Logistic Regression comparisons. 3 (16.7%) Glimmix analyses revealed significant differences when LOCF_Logistic Regression did not, while 2(11.1%) LOCF_Logistic Regression revealed a difference when Glimmix did not. Glimmix and LOCF_Logistic Regression were evenly split (9[50%]) in the number of comparisons yielding a lower significant difference as compared to the other.
Discussion: Different results were found between the ETRANK, MMRM and LOCF analyses of HAM-D_{17} change from baseline. ETRANK demonstrated advantages in signal detection compared to MMRM, perhaps explained by the former method of incorporating reasons and patterns of discontinuation. In this data set, MMRM had no advantage in detecting drug-placebo differences over the conventional LOCF method. No signal detection advantage likewise was found for the Glimmix MMRM method compared to LOCF_Logistic Regression analyses of remission rates.

82. Children of Depressed Mothers in an Urban Primary Care Population

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Sponsor: Myrna Weissman

Background: Several studies have shown that children of depressed parents are at increased risk for various psychiatric disorders and have higher levels of impairment in school and social functioning. Less is known about children from urban, low-income families. Community surveys consistently indicate that lower income samples are at greater risk for mental disorders. Our sample comes from an urban primary care practice serving low-income, predominantly immigrant Hispanic families from the Caribbean islands and Central America, with adults who speak primarily Spanish.
Methods: Thirty-five mothers (probands), selected from a larger sample of patients in an urban primary care practice, were classified into 2 groups based on the SCID-NP: depressed mothers had at least one lifetime episode of DSM-IV major depressive disorder (MDD) of at least 4 weeks duration and non-depressed mothers had no lifetime history of MDD. Up to 3 children per family aged 8 to 20 years were diagnostically assessed with the K-SADS-PL, administered separately to each child and mother as the informant by bilingual interviewers. Final diagnoses for mothers and children were based on the best estimate (BE) diagnostic procedure. The diagnostic interviewers and the BE were blind to the diagnostic status of the mothers. We also administered the Social Adjustment Inventory for Children and Adolescents (SAICA) and obtained information about mental health treatment. Results about mental health treatment and other measures will be presented at the meeting.
Results: Fifty-eight children from the 35 families were included in the study: 26 children of 16 depressed mothers and 32 children of 19 non-depressed mothers. The families in the sample were poor and many mothers had not completed high school. Age, sex and ethnicity distribution did not significantly differ between the two groups. Mean age of the children was 13.4 (+/- 3.0) years and 88% were Hispanic. Although there were more boys in the depressed proband group (61.5% vs. 43.8%), this difference did not reach statistical significance. Children of depressed mothers had significantly higher rates of depressive disorders (34.6% vs. 6.3%, p = .006) and disruptive behavior disorders (46.2% vs. 18.8%, p = .025), in particular attention deficit-hyperactivity disorder (26.9% vs. 6.3%, p = .031) and oppositional defiant disorder (30.8% vs. 6.3%, p = .014). While the overall rates of anxiety disorders did not significantly differ between the two groups, children of depressed mothers had significantly

higher rates of separation anxiety disorder (23.1% vs. 0, p = .006). Rates of substance use disorders did not significantly differ between the two groups. Children of depressed mothers also had significantly lower psychosocial functioning than children of non-depressed mothers. The mean Global Assessment Scores (GAS) were 63 vs. 78 (p = <.001) and the overall SAICA scores were 1.82 vs. 1.60 (p=.008), respectively. SAICA scores range from 1 to 4 and higher scores reflect lower functioning. As measured by the SAICA, children of depressed mothers had more school problems (1.58 vs. 1.32, p = .034), more spare time problems (1.65 vs. 1.22, p = .005), more sibling relationship issues (1.76 vs. 1.38, p = .033), lower overall home functioning (1.62 vs. 1.39, p = .032), and more overall problems (1.50 vs. 1.22, p = .014), compared to children of non-depressed mothers.
Discussion: These findings in children of depressed mothers coming to a primary care clinic are similar to findings in children of depressed mothers coming for psychiatric treatment. These results have implications for prevention and early intervention strategies in primary care practices serving low-income populations.

83. The DID Anhedonia Rating Scale: Results of the First Validation Study
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Sponsor: Fridolin Sulser

Background: Anhedonia has been considered to be a core symptom of depression since the term was coined by Ribot in 1896. Conceptually, it has been considered to be a state or personality variable, trait or symptom, or both. With the advent of diagnostic schedules, inquiry about anhedonia was limited to questions about loss of interest in activities and the ability to experience pleasure was considered a derivable variable.
Methods: The DID anhedonia scale assesses eight domains of pleasurable experience: Accomplishment, Personal Interests and Hobbies, Social Activities-Friends, Social Activities-Family, Sensory Experience, Appetite and Pleasure-Food, Appetite and Pleasure-Sexual Activity, and Spiritual or Religious Experience. Subject answers were not dependant on the recurrence of specific behaviors within a domain; they could make their rating based on similar activities within the domain on a subsequent week. An unanchored global anhedonia question was asked at the beginning and a similar but anchored question was asked at the end of the interview. For each item, interest and pleasure were assessed separately. A semi-structured interview with suggested questions was provided to the interviewers and the answers were scored according to the GRID format with frequency and severity considered separately. The standard used for comparison was the Bech sub-scale of the Hamilton Depression Rating Scale which was administered after the anhedonia scale. Item response analysis was used to evaluate the responses to each item. This technique assesses the relationship between each item score (0-4) and the total BECH score. This analysis provides a measurement of the sensitivity of responses within an item at different levels of disease severity. The correlations between individual item scores and the total BECH score were also calculated. 79 asymptomatic to severely depressed subjects were recruited at 5 research sites and evaluated by trained interviewers.
Results: The item correlations with the total Bech score were: the anchored global anhedonia items (interest and activity) 0.8, Accomplishment, Hobbies (interest), Social Activities-Friends (interest) >0.7 and Social Activities-Family (interest) 0.69. Accomplishment, Hobbies, Social Activities-Friends, and Social Activities-Family had good discrimination across the range of disease severity. Sensory Experience, Food and Appetite, Sexual Activity, and Spiritual Experience only discriminated at severe levels of depression. In general interest items performed better than the pleasure items and the anchored global item was superior to the unanchored question.
Discussion: The ability to experience pleasure is a core symptom of depression which is not directly assessed by either the Hamilton or Montgomery Asberg depression rating scales. The neurobiology of depression suggests that there may be an intimate relationship between the disease and both interest and pleasure. We have shown a clear relationship between depression and anhedonia in eight domains of pleasure as well as in a global item. Four of these items appear to be sensitive across a wide range of severity; four domains appear to be affected only when there is severe depressive symptomatology. The addition of an anhedonia item or scale may improve the precision of clinical trial research of affective disorders.

84. Passive Avoidance Learning and Response Reversal During Tryptophan Depletion
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Sponsor: Daniel Pine

Background: Abnormalities of serotonergic systems have been reported in depression and antisocial behavior. A recent study reports abnormal reward processing in depression[1], while neurocognitive studies in psychopathy demonstrate abnormalities of punishment processing and response reversal. Tryptophan depletion (TD), a procedure which transiently lowers CNS serotonin levels, permits investigation of the impact of lowered serotonin levels on cognitive processing. Prior work suggests reward processing deficits during TD [2], while reports of behavioral effects on response reversal deficits have been conflicting [3-5]. Based on prior studies, we hypothesized that reductions of serotonin via tryptophan depletion would impair reward processing and response reversal, while leaving punishment processing intact. To explore this hypothesis we administered the passive avoidance and probabilistic response reversal tasks during tryptophan depletion.
Methods: 36 healthy volunteers were screened and enrolled at the NIMH. Participants with a personal history or first degree family member with depression were excluded. Participants were administered 31.5 gm of amino acids (without tryptophan) or placebo. Five hours after capsule ingestion, participants performed the passive avoidance and probabilistic response reversal tasks. During the passive avoidance task participants were presented with 12 different two digit numbers. Responses to half of the numbers (CS+) resulted in a gain of points, while responses to others (CS-) caused a loss of points(+/- 1, 400, 800, 1200, 1600, 2000). Participants learned to respond to the rewarded stimuli and to withhold responses to the punished stimuli. During the probabilistic response reversal task [6], participants were presented a choice between pairs of colored objects on a screen. One object in each pair was rewarded with a probabilistic contingency of either 100% or 80%, while selection of the other object was punished with a 100% or 80% probabilistic contingency through loss of points. Part way through the task, the rewarded and punished objects in each pair reversed, and participants had to learn to reverse their response to gain points.
Results: During the passive avoidance task, participants who underwent tryptophan depletion committed more omission errors (failure to respond to a rewarded stimulus) than those receiving placebo. There was no difference in passive avoidance errors (inappropriate responding to punished stimuli). On the probabilistic response reversal task, there were no significant differences between tryptophan depletion and placebo groups in errors during either the acquisition or reversal phases for either contingency.
Discussion: Our finding of decreased sensitivity to reward is in line with prior reports of impaired discrimination of expected reward during tryptophan depletion [2]. Anhedonia, the clinical symptom of decreased sensitivity to reward seen in depression, may result from such altered serotonergic modulation of reward processing. The find-

ings do not support suggestions that serotonin plays a critical role in either punishment processing or response reversal. 1. Keedwell, P.A., et al. In Press, Corrected Proof; 2. Rogers, R.D., et al. Neuropsychopharmacology, 2003. 28(1): p. 153-62; 3. Rogers, R.D., et al. Psychopharmacology (Berl), 1999. 146(4): p. 482-91; 4. Evers, E.A., et al. Neuropsychopharmacology, 2005. 30(6): p. 1138-47; 5. Murphy, F.C., et al. Psychopharmacology (Berl), 2002. 163(1): p. 42-53; 6. Budhani S. et al.

85. Cognitive Performance in Bipolar Disorder: A Processing Efficiency Account

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Sponsor: Robert Kowatch

Background: Bipolar disorder is characterized by clinically significant impairment of attention and memory. It is unclear whether these represent formal cognitive deficits (an inability to perform accurately) or decreased processing efficiency (e.g., psychomotor slowing). We hypothesized that patterns of sustained attention and recognition performance in bipolar disorder were consistent with processing efficiency theory. This theory predicts that heightened mood states reduce the processing capacity of working memory available to perform cognitive tasks and increase on-task effort or strategy use to achieve adequate performance.
Methods: Twenty-five manic bipolar, 23 euthymic bipolar, and 28 healthy comparison participants were group matched for demographics and compared on a continuous performance task and verbal recognition memory test. Participants were also administered a variety of symptom-rating scales.
Results: Only the manic group demonstrated significant deficits in sustained attention and recognition accuracy. However, both the manic and euthymic groups slowed reaction time in an attempt to maintain high performance accuracy (i.e., increased effort by trading speed for accuracy). When reaction time was statistically controlled to equate the groups on effort/strategy use, the manic group continued to demonstrate a sustained attention deficit.
Discussion: Consistent with processing efficiency theory, these findings suggest that bipolar patients sustain attention and recognize verbal information through relatively greater effortful control at the expense of processing efficiency. Manic symptoms may reduce the capacity for control and impair attention and memory. Problems with processing efficiency are viewed as primary trait characteristics of bipolar disorder that may be overlooked by traditional error-based assessments. The predictions of processing efficiency theory with respect to bipolar disorder are discussed.

86. A Pilot Trial of Escitalopram for Depression in Perimenopausal Women

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Sponsor: Alan Gelenberg

Background: The perimenopause is a time of increased risk of depression in women, and it is often characterized by disruptive somatic symptoms, such as hot flashes. Epidemiological data support a higher risk of depression for women during the perimenopause. Hormone replacement therapy (HRT) has become increasingly controversial in light of the findings of the Women's Health Initiative study. Overall, results do support general use of HRT in women for prevention of cardiovascular disease, and indications for HRT have become more controversial since this study was released. Many women would prefer non-hormonal treatment options for the mood and somatic symptoms associated with perimenopause. Antidepressants are a class of medications that health care providers are likely to utilize for perimenopausal women instead of HRT. Fluoxetine, paroxetine, venlafaxine, and citalopram have all been utilized in studies for the treatment of vasomotor symptoms of perimenopause. Citalopram also appears promising as treatment for mood in the perimenopause. Our objectives were to assess the efficacy of escitalopram in women with perimenopausal depression and to evaluate the impact of the treatment on both mood and somatic symptoms of perimenopause.
Methods: This study was an open-label 8-week trial of escitalopram for perimenopausal depression and somatic symptoms. Eligible women were 40 years old or older with perimenopausal symptoms of at least 3 months duration, including irregular periods and/or hot flushes, and who were not were currently using hormone replacement therapy or antidepressants. Patients met criteria for a major depressive episode, verified with the Structured Clinical Interview for DSM-IV (SCID). Subjects received escitalopram 10 mg per day. The dosage could be titrated to a maximum of 20 mg. The primary efficacy measure for depression was the Hamilton Rating Scale for Depression (HRSD). The Greene Climacteric Index (GCI) was completed at each visit to quantify somatic symptoms of perimenopause. Rating scales were administered biweekly.
Results: Of 18 enrolled subjects, fifteen were included in an intent to treat analysis, with at least two time points of data. Of the 3 subjects not included in the analysis, two were consented for the study but opted not to participate and did not start medication, and one dropped out of the study prior to the second visit due to a complaint of increased anxiety after three days of medication. All participants were assessed at baseline. The mean score for the HRSD at baseline was 18.13 and the mean score at the second time point was 8.07. An ANOVA was calculated and the pretest posttest means were found to be significantly different ($F = 59.66$, $p < 0.01$). Fifteen participants were scored with the GCI on at least two time points. The mean score for the GCI at baseline was 26.4 and the mean score at the second time point was 10.2. An ANOVA was calculated and the pretest posttest means were found to be significantly different ($F = 34.75$, $p < 0.01$).
Discussion: In this open-label study, escitalopram was well tolerated and appeared efficacious for depression in perimenopausal women, as well as efficacious for the somatic symptoms of perimenopause. Limitations to this study included small sample size and lack of placebo control group. These findings support controlled studies with escitalopram in perimenopausal women, especially considering the increasingly controversial role of HRT in this population.

87. Modafinil in the Treatment of Bipolar Depression: A Placebo-Controlled Trial

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Background: Modafinil is currently F.D.A. approved for daytime sedation and somnolence associated with narcolepsy, obstructive sleep apnea and shift work sleep disorder. This study was conducted to further evaluate the efficacy and safety of modafinil in the treatment of bipolar depression.
Methods: This study was a 6-week, randomized, double-blind, placebo-controlled evaluation of modafinil (100 mg qd X 1 week, 200 mg qd x 5 weeks; mean dose 177 mg) in subjects with bipolar I or II depression that was inadequately responsive to mood stabilization +/- adjunctive antidepressant therapy. The primary outcome measure was baseline to endpoint change in the Inventory for Depressive Symptoms (IDS, Rush et al., 1988); this is a 30-item clinical rating scale that rates severity of depression (score range 0-90). Secondary outcome measures including baseline to endpoint change on the Young Mania Rating Scale (Young et al., 1978), Epworth Sleepiness Scale (Johns et al., 1991), and Fatigue Severity Scale (Krupp et al., 1989). 87 subjects were randomized and the intent to treat efficacy analysis was performed on subjects who received 1 dose of study drug and 1 rating

post randomization. 41 subjects were randomized to modafinil and 44 subjects were randomized to placebo. Baseline and endpoint differences were analyzed by analysis of variance. Time course of antidepressant response was analyzed my mixed effects model.
Results: There were no difference in mean age, gender distribution, BPI subtype, or percent rapid cycling in the modafinil (MOD) and placebo (PLC) groups. There was no difference in mean baseline depression IDS score (MOD= 31.68, PLC= 32.49). There was a greater baseline to endpoint change in MOD vs. PLC subjects (MOD=20.48, PLC= 26.52; ANOVA baseline; F=1.11,p=0.29, end: F=4.39, p=0.039, med*time: F=2.99, p=0.09). Between the two groups, the IDS change from baseline was significantly greater in the MOD group by week 2; this difference was maintained for the duration of the study. 44% of MOD subjects (endpoint IDS 20.48) vs. PLC subjects (endpoint IDS 26.52; subjects had a 50% or greater improvement in the IDS score vs 22% of PLC subjects (X2 = 4.31, p=0.038). There was no difference in baseline (MOD= 1.51, PLC= 2.38) or endpoint (MOD=2.69, PLC= 3.7) YMRS scores (ANOVA baseline: F=2.35, p=0.13; End: F=1.01, p=0.32; med*time: F=0.04, p=0.85). The most common side effect was headache (MOD= 12.3%, PLC= 2.3%). During the course of the study, there were 2 hospitalizations for mania (1 MOD/ 1 PLC), 1 hospitalization for depression (1 MOD), 1 depression exacerbation (1 PLC), and 5 occurrences of treatment emergent hypomania (1 MOD/ 4 PLC).
Discussion: This data suggests that adjunctive modafinil 100-200 mg qd may improve depressive symptoms in bipolar disorder. Additionally, this data suggests that adjunctive modafinil does not destabilize bipolar depression. Headache appears to be the most common side effect. Open trial continuation phase data is currently being analyzed.

88. Epigenetic Determinants of GAD1/GAD67 (2q31.1) Expression in Human Prefrontal Cortex

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Sponsor: Edward Jones

Background: Glutamic acid decarboxylase (GAD) is a key enzyme for gamma-amino butyric acid (GABA) synthesis and inhibitory interneuron function. Single nucleotide polymorphisms residing in regulatory sequences of GAD1, the gene encoding the 67kDa isoform of GAD, confer genetic risk for childhood-onset schizophrenia (Addington et al., 2005; Mol Psychiatry 10:581) and some cases of bipolar disorder (Lundorf et al., 2005; Am J Med Genet B 135: 94). Furthermore, alterations in GAD1 protein and/or mRNA levels were repeatedly observed in postmortem brain of subjects diagnosed with schizophrenia, bipolar disorder or autism, suggesting that GAD1 plays a key role in the molecular pathology of several major psychiatric disorders.
Methods: Nothing is known about molecular mechanisms governing transcriptional regulation of GAD1 in human brain. The methylation of specific lysine residues located at the amino-terminal tails of the nucleosome core histones H3 and H4 is increasingly recognized as a molecular imprint involved in the long-term regulation of gene expression (Sims et al., 2003; Trends Genet 19:629). Specifically, a combinatorial set of histone methylation marks is differentially regulated at sites of open chromatin and potential transcription, in comparison to closed or silenced chromatin. We developed a modified native chromatin immunoprecipitation assay (NChIP) to map, in postmortem brain, histone methylation profiles at defined genomic regions (Stadler et al., 2005; J. Neurochem, in press). Here, we use NChIP to determine levels of histone H3 methylation (H3-lysine 4, H3-lysine 27) at GAD1 promoter sequences in human prefrontal cortex collected across a wide age range from midgestation to 90 years old.
Results: We observed striking differences in the distribution of open- (H3-trimethyl-lysine 4) and closed (H3-trimethyl-lysine 27) chromatin associated histone methylation across a 20kB region surrounding the GAD1 transcription start site. Furthermore, GAD1-associated histone H3-lysine 4 methylation was differentially regulated in prenatal and infant prefrontal cortex, in comparison to adults.
Discussion: Our data suggest that differential histone methylation at the GAD1/GAD67 promoter could be involved in the developmental regulation of prefrontal GAD1/GAD67 expression. Acknowledgements: Postmortem brain tissue was obtained through the Brain and Tissue Banks for Developmental Disorders, University of Maryland and University of Miami (NICHD contract # NO1-HD-8-3284). Adult tissue samples were obtained from the brain collection at Center for Neuroscience, University of California at Davis (Dr. E.G. Jones). Supported by NICHHD and NIMH.

89. Asenapine, a Novel Psychotherapeutic Agent with Efficacy in Positive and Negative Symptoms During Acute Episodes of Schizophrenia: A Randomized, Placebo- and Risperidone-Controlled Trial

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Sponsor: Steven Potkin

Background: The promise of improved efficacy and tolerability of the atypical antipsychotics compared with typical or first-generation antipsychotics remains only partially fulfilled. As consensus grows around the need to treat specific symptom domains of schizophrenia, such as negative and cognitive symptoms, the shortcomings of available medications become evident. In particular, there are no treatments with proven efficacy for primary negative symptoms (American Psychiatric Association Practice Guideline for the Treatment of Patients With Schizophrenia, 2004). Negative symptoms are burdensome for family members and contribute to poor long-term treatment outcomes for patients with schizophrenia. Asenapine, a novel psychotherapeutic agent, reached 75% occupancy of the D2 receptor in previous studies in schizophrenic subjects at a sublingual dose equivalent to 6 mg/day (Potkin et al, ACNP 2003). In the current trial, a dose of asenapine 5 mg BID was chosen to test its efficacy, tolerability, and safety versus placebo and risperidone 3 mg BID in subjects with an acute exacerbation of schizophrenia.
Methods: Subjects with a diagnosis of schizophrenia and a baseline Positive and Negative Symptom Scale (PANSS) score >60 were randomized to receive asenapine 5 mg BID, oral risperidone 3 mg BID, or placebo (BID) for 6 weeks. Weekly efficacy evaluations were made using the PANSS. Data was analyzed by comparison of least-squares means based on an ANOVA model of the intent-to-treat population (ITT), using a last-observation-carried-forward approach.
Results: The ITT population consisted of 58 asenapine, 56 risperidone, and 60 placebo subjects. Improvement from baseline to week 6 in mean PANSS total score was significantly greater with asenapine (-15.7; $p<0.05$) than with placebo (-4.3); risperidone (-9.7) was numerically greater than with placebo. PANSS positive subscale scores improved significantly with asenapine and risperidone versus placebo (both $p<0.05$ at endpoint). Improvements in PANSS negative symptom subscale scores were significantly greater with asenapine versus placebo at Weeks 2 to 6 (Week 2, $p<0.05$; Weeks 3 to 6, $p<0.01$) and versus risperidone at Weeks 3 to 6 ($p<0.05$). All treatments were well tolerated. Clinically significant weight gain (a gain of greater than or equal to 7% of baseline body weight) occurred significantly more often with risperidone (8/47, 17.0%, $p <0.05$) than with placebo (1/54, 1.9%), while clinically significant weight gain for asenapine (2/46, 4.3%) was comparable to that with placebo.
Discussion: In this trial, asenapine 5 mg BID showed superior efficacy to placebo at weeks 2-6 for total PANSS scores, PANSS positive subscale, and PANSS negative subscale scores; better efficacy than risperidone 3 mg BID on the PANSS negative subscale, and good tolerability without clinically significant weight gain. Asenapine has a chemical structure and receptor signature distinct from the currently available atypical antipsychotics (Shahid et al; ACNP 2005). In partic-

ular, asenapine has higher affinity for the 5HT2C, 5HT2A, 5HT6, D3, alpha1 and alpha2 receptors than for the D2 receptor and compared with risperidone. Additional clinical trials are underway to further evaluate the efficacy of asenapine in the treatment of schizophrenia, including in patients with predominant negative symptoms.

90. Training for Assessment of Negative Symptoms of Schizophrenia Across Languages and Cultures Utilizing the Negative Symptom Assessment Scale

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Sponsor: John Davis

Background: The Negative Symptom Assessment Scale (NSA) is a 16-item clinician rated instrument for rating the negative symptoms of schizophrenia. Its reliability and concurrent validity with similar instruments has previously been assessed in English speaking raters (Axelrod and Alphs, 1993; Axelrod, Goldman and Alphs, 1993). The NSA compared favorably to the BPRS retardation factor in its ability to detect change in schizophrenic subjects (Eckert et al, 1996). In large scale, international, multi-center clinical trials, agreement among raters with respect to quantification of negative symptoms may be complicated by language differences and variations in cultural interpretation of symptoms. Lack of agreement among raters on measurement technique is a source of non-specific variance in ratings that may diminish statistical power and increase the number of subjects required for a valid study.
Methods: The objective of this analysis was to assess whether high levels of agreement among raters across multiple nationalities and languages could be achieved in measurement of negative symptoms with the NSA. 120 raters from the United States and 175 raters from 18 other countries were trained to rate the NSA by viewing at least one training lecture and viewing and rating at least one videotaped, semi-structured NSA interview of a schizophrenic patient, followed by detailed feedback on the proper rating methods. Subsequently, raters were evaluated on their rating of an additional videotaped, semi-structured NSA interview of a schizophrenic patient. The raters consisted of two non-overlapping cohorts of multi-site international clinical trial investigators who were generally unfamiliar with the NSA prior to the training. The a priori measure utilized to evaluate acceptable agreement among raters was to score within one point of the modal score of their cohorts on at least 80% of the 16 NSA items. The same cohorts of raters were concurrently trained to rate the Positive and Negative Symptom Scale (PANSS) by a method analogous to that by which the NSA was taught. However, most raters had had previous training and experience in administering the PANSS to patients.
Results: 85 of 90 US raters (94%) and 174/180 (96%) of raters from other countries met the a priori criteria for acceptable agreement in rating of the NSA. (US vs. non-US X2=.76, DF=1, p=.38). In contrast, 104 of 120 US raters (86%) and 170 of 175 raters from other countries (97%) met the a priori criteria for acceptable agreement in rating of the PANSS. (US vs. non-US X2=11.8, DF=1, p=.0009).
Discussion: High levels of agreement in rating the NSA appear to be feasible among clinical trials raters from multiple countries. Training of multinational cohorts in rating the NSA appeared to be at least as successful as that of US cohorts. Training in rating of the NSA appeared at least as successful as that for the PANSS.

91. Insulin And Igf-1 Reverse Mitochondrial, Energy Metabolism, Proteasome, And Neuronal Plasticity Gene Decreases Of Schizophrenia In An Mrna-Based Screen For Antischizophrenia Agents

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Background: Using hippocampal dentate granule neurons captured from multiple groups of schizophrenic patients and control cases, we have identified decreases in large clusters of CNS genes that encode for mitochondrial oxidative energy metabolism (isocitrate, lactate, malate, NADH and succinate dehydrogenases; cytochrome C oxidase and ATP synthase), protein turnover (proteasome subunits and ubiquitin), neurite outgrowth, cytoskeletal proteins, and synapse plasticity. These changes were not observed in bipolar disorder or non-psychotic major depression (Altar et al, 2005). Decreases in many of these same genes in diabetic skeletal muscle (Yechoor et al, 2002) and their upregulation in human skeletal muscle by insulin (Rome et al, 2002) suggested to us (1) that schizophrenia could in part result from deficiencies in insulin-dependent signal transduction pathways, and (2) that a novel treatment for schizophrenia could occur by a coordinate up-regulation of the genes that encode for these biochemical deficits in schizophrenia.
Methods: Using the SH-SY5Y human neuroblastoma cell line, we found that insulin and IGF-1 produced highly significant, reproducible, and reciprocal changes in a large number of the schizophrenia-associated genes. No such changes occurred with IGF-2, BDNF, clozapine, haloperidol, or 60 other CNS-active ligands. Among the genes that decreased in either schizophrenia group, 317 were also increased by insulin in the SH-SY5Y cells. A statistical evaluation by EASE analysis segregated these 317 schizophrenia-insulin genes into 8 highly significant biochemical classes (EASE p values of 10-4 to 10-10). Fourteen of these genes were selected to represent the mitochondrial, glucose metabolism, Krebs cycle, proteasome, ubiquitin, synaptic, and neurotransmitter signaling functions identified by EASE and other considerations. These 14 genes, and 2 control genes that did not change in schizophrenia or to insulin or IGF-1, were incorporated into a nuclease protection-based multiparameter high-throughput screen (MPHTS). The effects of exposing the SH-SY5Y cells to 25 uM concentrations of 2339 pharmacologically active small molecules were determined by luminance detection.
Results: Insulin, IGF-1, and about 0.5% of the 2339 small molecules produced highly significant and reproducible changes in 9-13 of the MPHTS genes. The changes were reciprocal to the changes found in schizophrenia. A single class of specific receptor antagonists blocked the multi-gene effects of the most active small molecules, but neither insulin nor IGF-1.
Discussion: Defective insulin signaling may predispose schizophrenic patients to type II diabetes and, in hippocampal and neocortical neurons, to psychiatric and cognitive symptoms. Insulin, IGF-1, and a distinct class of small molecules can increase in a human neuronal cell line the same genes that are decreased in schizophrenia. The inability of conventional antipsychotic drugs to affect most of these genes suggests that the MPHTS drug screen may identify novel antischizophrenia agents that more directly address the root causes of schizophrenia.

92. A Double-Blind Randomized Trial of a Single Dose of Placebo, Haloperidol, and Risperidone in Healthy Volunteers

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Sponsor: Robert Buchanan

Background: The role of the effect of antipsychotics on primary negative symptoms is controversial. Prior studies have not assessed the effect of these drugs in normal controls in a double-blind paradigm.
Methods: Thirty-two normal volunteer controls recruited from advertisments in the hospital were included. Double-blind placebo controlled trial of single dose of haloperidol (5 mg) and risperidone (2.5 mg) in healthy subjects. Motor variables and observer-rated negative symptoms were assessed after 3-4 hours and subjective negative symptoms and drowsiness, after 24 hours.

Results: Neither of the two drugs caused significant motor EPS after administration. Initially, haloperidol caused significantly more negative signs and symptoms than placebo in the following measures: SANS ($p=0.044$), and two scales of self-rated negative symptoms: Bitter and Jaegers total score ($p<0.001$) and an Analogue Scale on subjective negative symptoms ($p=0.004$) Risperidone caused significantly more negative signs and symptoms than placebo in: BPRS, SANS, Bitter and Jaegers total score and the Analogue Scale on subjective negative symptoms (all with $p<0.001$). After controlling for drowsiness risperidone but not haloperidol produced more negative symptoms than placebo as measured on the BPRS and SANS (both $p<0.02$). Significance was lost for the subjective negative symptoms with both drugs after controlling for drowsiness.
Discussion: Single doses of both haloperidol and risperidone produce negative symptoms, rated in both observer-rated and self-rated or subjective scales in normal controls. Drowsiness may be an important confounding factor when assessing negative symptoms in antipsychotic trials.

93. Corticotropin-Releasing Factor (CRF) Impairs Sensorimotor Gating In The Sprague-Dawley Rat: Reversal By Clozapine And Raclopride
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Sponsor: Richard Shader

Background: Exposure to social stress is associated with the onset and relapse of schizophrenia symptoms, including deficits in sensorimotor gating. Impaired sensorimotor gating can be measured by a loss of normal prepulse inhibition of the startle response (PPI), which is associated with hyperactive central dopaminergic, serotonergic or noradrenergic systems. CRF is released during stress and regulates levels of monoamines involved in PPI. Moreover, elevated levels of CRF have been reported in CSF of schizophrenia patients, suggesting that CRF hyperactivity could trigger schizophrenia symptoms.
Methods: The present study assessed the effect of centrally administered human/rat CRF (1.0 μg or 3.0 μg, i.c.v.) on percent PPI in male Sprague-Dawley rats (Harlan, San Diego, CA) 40 min and 24 h after CRF infusion. CRF- induced grooming and locomotion were also assessed following drug infusions to ensure the successful delivery of the peptide into the CNS. Next, we tested the ability of clozapine (5 and 10 mg/kg, i.p.), an atypical antipsychotic, and raclopride (0.05 and 0.1 mg/kg, s.c.), a selective D2-like receptor antagonist, to reverse CRF-induced PPI deficits. Clozapine was administered i.p. either 20 min before or 15 min after the CRF infusion, while raclopride was administered s.c. 10 min before or 25 min after the CRF infusion.
Results: CRF produced a dose-dependent disruption of PPI compared to baseline, and this effect was absent the next day. Both distance traveled and time spent grooming increased significantly following the administration of both CRF doses. In contrast, mean startle response to pulse stimuli was not altered. Clozapine pretreatment dose-dependently increased CRF-induced PPI disruption, with complete reversal at the highest dose of clozapine. However, CRF-induced PPI disruption was only partially reversed by the highest dose of raclopride. Neither drug altered PPI when administered alone, nor was either drug effective when administered after CRF infusion.
Discussion: The results demonstrate that CRF disrupts PPI in a dose-dependant manner, and this disruption is blocked by clozapine pretreatment. The partial effect of raclopride pretreatment suggests that activation of D2-like receptors might play a role in the mechanism underlying CRF- or stress-induced sensorimotor gating deficits.

94. Type of Symptom Remission and Treatment Outcomes in the Long-Term Treatment of Patients with Schizophrenia
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Sponsor: John Kane

Background: This prospective study examined the relationships between type of symptom remission and type of treatment outcomes during the long-term treatment of patients with schizophrenia.
Methods: We used data from a large 3-year multi-site naturalistic study of patients with schizophrenia in the United States in which participants were assessed at enrollment and at 6 or 12-month intervals thereafter. We identified four mutually exclusive groups of patients based on their type of symptom remission: (a) remission of psychotic symptoms (positive and negative), as defined by the Schizophrenia Working Group expert consensus criteria using the Positive and Negative Syndrome Scale, (b) remission of depressive symptoms, defined as a score of ≤9 on the Montgomery-Asberg Depression Rating Scale, (c) remission of both psychotic and depressive symptoms, and (d) non-remitted status on both depressive and psychotic symptoms. A broad range of outcome domains was assessed with validated measures (e.g., occupational functioning, safety in the community, substance use, activities/relationships, mental health resource utilization, life satisfaction, quality of life). Effect sizes were calculated to assess the differential impact of each remission type relative to the non-remitted group on each outcome variable at enrollment, at the end of Year 1, 2, and 3 of the study, and across the 3-year study.
Results: Across the 3-year study, remission of both psychotic and depressive symptoms was accompanied by best treatment outcomes in numerous domains. Compared to remission of psychotic symptoms, remission of depressive symptoms was more related (greater effect sizes) to better mental health functioning, greater life satisfaction, better family relationships, greater medication adherence, lower likelihood of seeking emergency psychiatric services, fewer alcohol-related problems, and a lower risk of being a safety concern in the community (suicidal thoughts, suicide attempts, violent behavior, being victimized). Remission of psychotic symptoms was more related to higher Global Assessment of Functioning (GAF) scores, better quality of life, and higher activity levels (social, daily, leisure, and productive activities).
Discussion: Remission of specific symptom domains appears to differentially contribute to distinct treatment outcomes. Treatments that are able to improve both psychotic and depressive symptoms are apt to provide greater therapeutic benefits and to impact more outcome domains.

95. PCP-Disrupted Social Interaction Paradigm in Rats: Differential Effects of Typical and Atypical Antipsychotics: Putative Role of α2-Adrenoceptor Antagonism?
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Sponsor: Marc Caron

Background: We need to create animal models of negative symptoms in order to help develop new compounds to meet medical need in schizophrenia (SCZ) patients. Administration of the NMDA-antagonist, phencyclidine (PCP), induces social withdrawal in rodents (Steinpreiss et al 1994, 1997). This can be attenuated by acute treatment with some neuroleptics (Corbett et al 1995, Sams-Dodd 1996, Golembo et al 2003). As a first step towards validating this paradigm we have tested an extensive gamma of reference neuroleptics in order to gain more insight into its clinical relevance, and into the pharmacological mechanisms responsible for activity.

Methods: Pairs of weight-matched unfamiliar rats receiving identical treatments were placed in a dimly lit circular arena, and videotaped for 10 minutes. Social behaviour (body and genital investigation, following, playing, partner grooming) was scored manually in The Observer for 5 minutes. Ethovision Pro quantified additional measures of activity. Test compounds were administered s.c. 45' prior to testing, PCP (3 mg/kg) was given s.c.15 mins prior to session start. The highest dose of the neuroleptics used had only a minor influence on locomotor activity in normal rats.
Results: Saline treated rats engaged in social interaction for approximately 50s per 5 mins in the low light and quiet conditions of the arena, this behaviour was reduced to about 5s following treatment with PCP. Typical neuroleptics Haloperidol (0.0025-0.04 mpk) and Pimozide (0.01-0.16 mpk) did not reverse the PCP induced deficits. Some atypical neuroleptics (* indicates significantly active doses) Clozapine 0.16, 0.63*, 2.5*, Risperidone 0.01, 0.04, 0.16*, Quetiapine 0.63, 2.5, 10*, and Ziprasidone 0.31, 1.25*, 5* were able to partially reverse PCP induced deficits. However, Olanzapine 0.04-0.63, Amisulpride 0.63-10, and Aripiprazole 0.16-2.5 were inactive. The anxiolytic Diazepam 0.08, 0.32, 1.25* and the non-specific $\alpha 2$ antagonist Idazoxan 0.16, 0.63, 2.5*, 5*, 10 were also active in this model.
Discussion: Any pharmacological intervention that improves social behaviour and/or negative symptoms may have important implications for long-term outcome in SCZ. The PCP-disrupted social interaction paradigm in rats appears to possess low-variability and good reproducibility. PCP 3mpk consistently reduced social interaction, without inducing hyperlocomotion or high levels of ataxia and stereotypy. This suggests that this dose of PCP models the negative rather more than the positive symptoms of SCZ. Typical neuroleptics were unable to restore social behaviour suggesting that D2-blockade alone is insufficient for activity. Some atypicals: Clozapine, Quetiapine, Risperidone and Ziprasidone partially reversed the effects of PCP. However, other atypicals: Olanzapine, Aripiprazole, and Amisulpride were inactive. This suggests that differential receptor occupancy profiles may provide a key to further elucidate the underlying pharmacological mechanisms. We demonstrated a putative role of $\alpha 2$ adrenoceptors and GABAa receptors in remediation of PCP-disrupted social interaction. An ideal drug in this paradigm would completely reverse the effects of PCP on social interaction without inducing significant side-effects. Active atypical neuroleptics only partially reverse the effects of PCP. Future basic research should therefore concentrate on the effects of novel mechanisms in this model in an attempt to reach the ideal. Further clinical work is also needed to better define the activity of antipsychotic and other drugs on negative symptoms in SCZ patients.

96. Predictors of Patient Satisfaction with Medication in Patients with Schizophrenia

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Sponsor: Michael Davidson

Background: A placebo-controlled study evaluating the effects of atypical antipsychotics in patients with a recent exacerbation of schizophrenia included an evaluation of patients' satisfaction and acceptability of risperidone and quetiapine, using the Medication Satisfaction Questionnaire (MSQ).
Methods: In a 2-phase, double-blind, multisite, international, 6-week study, patients were randomized to risperidone, quetiapine, or placebo monotherapy for the first 2 weeks. Investigators were then permitted to prescribe additional psychiatric medications as necessary during the 4-week additive-therapy phase. Patients were maintained on their day-8 dose for the remainder of the study. In addition to standard efficacy and safety assessments, patients self-rated their satisfaction with treatment using the MSQ, a 7-point scale ranging from extremely dissatisfied (score of 1) to extremely satisfied (score of 7). Univariate linear regression followed by step-wise multiple linear regression analyses tested the association between medication satisfaction and several factors (predictors), including efficacy and safety measures at weeks 2 and 6 (e.g., PANSS and HAM-D change scores and adverse events), demographic characteristics (age and sex), and treatment with risperidone or quetiapine.
Results: Reductions in mean PANSS total scores were significantly greater with risperidone than placebo ($P < 0.01$) or quetiapine ($P \leq 0.001$) at the monotherapy endpoint and significantly greater with risperidone than placebo at the additive-therapy endpoint; differences between quetiapine and placebo were not significant. Mean (±SE) scores on the MSQ at the monotherapy and additive-therapy endpoints were 5.2 ± 0.1 and 5.4 ± 0.1 in the risperidone group, 4.7 ± 0.1 and 4.8 ± 0.1 in the quetiapine group, and 4.5 ± 0.2 and 4.6 ± 0.2 in the placebo group. At both endpoints, significantly greater medication satisfaction was shown by the risperidone group than the quetiapine ($P < 0.01$) or placebo ($P < 0.001$) group; satisfaction with quetiapine treatment was not significantly different from placebo ($P > 0.1$). At the monotherapy endpoint, significant predictors of medication satisfaction according to the univariate linear regression model were PANSS total change scores, change scores on the 5 PANSS factors (positive symptoms, negative symptoms, disorganized thoughts, uncontrolled hostility/excitement, and anxiety/depression), and treatment with risperidone. According to the stepwise multiple linear regression model, the significant predictors were change scores on 3 PANSS factors (positive symptoms, hostility/excitement, and anxiety/depression), age, and treatment with risperidone. Safety issues, such as EPS, sedation, or prolactin elevation, were not predictive of dissatisfaction. Overall, similar findings were seen at the additive-therapy endpoint.
Discussion: The MSQ data suggest that the significantly greater patient satisfaction with risperidone than quetiapine or placebo may be related to the greater symptom reduction associated with risperidone.
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97. Targeting the Cystine-Glutamate Antiporter to Attenuate the Behavioral and Neurochemical Effects in the Phencyclidine Rodent Model of Schizophrenia

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Background: Earlier findings have demonstrated that PCP-induced working memory deficits and glutamate release in the prefrontal cortex are blocked following stimulation of group 2/3 mGluRs. The present study examined the hypothesis that increasing the activity of cystine-glutamate exchange, which has previously been shown to provide stimulate group 2/3 mGluRs, would block phencyclidine (PCP)-induced behavioral and neurochemical changes.
Methods: Behaviors examined in the present study include PCP-induced working memory deficits, assessed from performance in a forced-delayed alternation t-maze task and PCP-induced social withdrawal, assessed by determining time spent in close proximity (20 cm) with an unfamiliar rat in an open field maze (100 x 150 cm). Rats included in the t-maze experiments were trained to perform a forced-delayed alternation task (10 trials/day) until performance exceeded 80% over three consecutive sessions.
Results: PCP administration (0-3 mg/kg, SC) dose-dependently decreased performance with the highest dose reducing accuracy to chance levels (mean + SEM: 48 + 3%). PCP-induced working memory deficits were attenuated in rats pretreated (1.5 hr) with the cysteine prodrug N-acetylcysteine (mean + SEM: 71 + 4%). Interestingly, the group 2/3 mGluR antagonist LY 341495 (0-1 mg/kg, IP) prevented NAC-induced reversal of working memory deficits produced by PCP (3 mg/kg, SC; mean + SEM from rats treated with LY 341495 + NAC + PCP: 50 + 10%). Rats included in the social interaction experiments were placed in a novel compartment (100 x 150 cm) with an unfamiliar rat for 10 minutes. The time spent in close proximity (< 20 cm) was measured using image tracking software (EthoVision, Noldus Inc.). Phencyclidine administration (3 mg/kg) significantly reduced social interaction relative to saline controls (mean +

SEM: 69 + 8.5 and 178 + 14 seconds, respectively), and this effect was also attenuated by N-acetylcysteine pretreatment (90 mg/kg, SC; mean + SEM: 195 + 26). Lastly, additional rats underwent microdialysis testing. PCP (3 mg/kg, SC) produced a significant increase in prefrontal glutamate levels that was blocked by pretreatment (1.5 hr) with the cysteine prodrug n-acetylcysteine (90 mg/kg, IP; NAC).
Discussion: Collectively, these data indicate that the behavioral and neurochemical effects of PCP that may be relevant for schizophrenia can be attenuated by targeting cystine-glutamate exchange using cysteine prodrugs. These findings are the first to identify cystine-glutamate exchange as a novel target in the development of experimental pharmacotherapies for schizophrenia.

98. NRG-1 Protein Levels in Patients with Schizophrenia, Bipolar Disorder and Major Depression

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Sponsor: Peter Whitehouse

Background: NRG-1, a pluripotent growth factor, is one of the most highly replicated susceptibility genes for schizophrenia recently implicated in bipolar disorder as well. However, the etiological pathways whereby alterations in NRG-1 may lead to the development of schizophrenia/bipolar are undetermined. Back signaling of NRG-1 is a potent biological route whereby the intracytoplasmic (IC) domain of NRG-1 translocates to the nucleus, influences gene expression and ultimately determines neuronal survival. In this report, we used extracted and cell fractionated protein from the prefrontal cortex of tissue available from the Stanley Foundation (Torrey et al., 2000) to assess the amount of NRG-1 IC protein present in the nucleus of normals and patients with major mental illness.
Methods: We used the semi-quantitative western blot analysis and the primary antibody to NRG-1 from Santa Cruz (cat # sc-348) that recognizes the c-terminal region of NRG-1. We normalized the NRG-1 levels by measuring b-actin on the same gels (Chemicon, cat # MAB1501).
Results: We found that normalized NRG-1 IC protein levels at 50 kDa vary with pH in normal controls in both the nuclear and the cytoplasmic fractions ($r=-0.57$, $p=0.03$ and $r=-0.63$, $p=0.01$ respectively). We were unable to detect an overall diagnostic difference in normalized NRG-1 IC 50 kDa isoform in the nucleus of the four groups examined (normals, schizophrenics, bipolars and depressed) using ANCOVA with pH as co-variate ($df=3, 53$; $F=0.46$, $p=0.70$). Furthermore, we were unable to detect a difference in NRG-1 protein levels in the cytoplasm, nor were we able to detect a difference in the nuclear versus cytoplasmic ratio of NRG-1 protein in any of the four diagnostic groups evaluated for both the 50 and the 57 kDa isoforms (all $p>0.05$).
Discussion: Our preliminary findings do not support the hypothesis that NRG-1 protein levels are altered in the nucleus of the cortical cells of patients with severe mental illness; however we are developing a NRG-1 ELISA to address this question with a more rigorous quantitative method. In previous studies, we found a relative increase in a specific form of NRG-1 mRNA (putative type I). Thus, development of NRG-1 antibodies that specifically detect the various types of NRG-1 (I-IV) may be more informative.

99. Alterations of Postsynaptic AMPA Receptor Trafficking in the Anterior Cingulate Cortex in Elderly Schizophrenics

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Sponsor: Margit Burmeister

Background: Manifestation of some of the cognitive symptoms in patients with schizophrenia might be linked to structural and functional abnormalities that have been described in the anterior cingulate cortex (ACC) in this illness. Ampakines, positive AMPA receptor modulators, improve cognitive function in schizophrenia, and enhancement of AMPA receptor-mediated currents by these compounds potentiates the activity of antipsychotics. We hypothesize that there is a defect in the insertion in the membrane and/or trafficking of the AMPA receptors, and that the substrate of this disturbance may involve dysregulation of critical protein-protein interactions in the postsynaptic cell. Recently, in vitro studies have revealed that dynamic rearrangement of AMPA receptors in and out of the synaptic membrane is mediated by specific interactions of a complex network of proteins at the postsynaptic density (PSD).
Methods: Using postmortem brain samples, we analyzed by western-blotting protein levels of the AMPA trafficking molecules NSF, GRIP1, ABP, and PICK1 (linked to vesicular trafficking of GluR2-containing AMPA receptors), SAP97 and RIL (mediators of GluR1-containing AMPA receptor trafficking) and stargazin (interacting with all four AMPA subunits and involved in lateral translocation of AMPA receptors and clustering at the synapse). Using the same samples, we quantified the interaction of specific dyads of AMPA subunits and related PSD proteins by co-immunoprecipitation, to explore changes of protein:protein interactions in AMPA trafficking pathways.
Results: Protein expression for some AMPA receptor subunits and related PSD proteins are abnormal in schizophrenia. The GluR1 and GluR3 AMPA subunits, SAP97, RIL, NSF, and PICK expression were all decreased in the ACC compared to controls and preliminary data indicate that some of their interactions are also affected in ACC in schizophrenia.
Discussion: Based on these data, we hypothesize that glutamatergic AMPA neurotransmission is compromised in schizophrenia affecting probably cognitive performance monitoring by the ACC, as AMPA-related PSD protein interactions mediate AMPA receptor trafficking and synaptic surface expression, and are essential for the signaling cascade associated with glutamate synaptic transmission. Supported by MH53327 and The Stanley Foundation.

100. AVE1625, a Cannabinoid CB1 Antagonist, as a Co-Treatment for Schizophrenia: Improvement in Cognitive Function and Reduction of Antipsychotic-Side Effects in Animal Models

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Background: Cannabinoids exert their biologic effects via CB1 receptors in the CNS and CB2 receptors on many immune cells. Cannabis use can mimic negative symptoms, attentional and working memory deficits seen in schizophrenia and can precipitate psychosis or relapse in vulnerable people. Endocannabinoids are distributed throughout the brain, with high levels in the prefrontal cortex and the hippocampus, and, via CB1, regulate neurotransmitter release. Preclinical and clinical evidence suggests that endocannabinoid tone is raised in schizophrenia patients. CB1 receptors have also been shown to be elevated in both the frontal cortex and anterior cingulate cortex of schizophrenia patients. Converging animal data using cannabinoids, CB1 antagonists and CB1 KO mice suggest that CB1 antagonists might improve the cognitive deficits associated with schizophrenia.
Methods: AVE1625((N-{1-[Bis-(4-chloro-phenyl)-methyl]-azetidin-3-yl}-N-(3,5-difluoro-phenyl)-methanesulfonamide) is a highly potent (Ki of 0.16-0.44 nM) and selective CB1 antagonist. Here we have tested the the effects of AVE1625 in a variety of animal models to assess its potential as a treatment for schizophrenia patients.
Results: We have demonstrated that AVE1625 improves cognition, particularly working memory, in a variety of rodent models. AVE1625 improved working memory performance in the rat hole

board (3 mg/kg) and 8-arm radial maze (1 mg/kg) tests. AVE1625 also reversed MK801-induced persistent latent inhibition in rats (1 mg/kg). AVE1625 (up to 10 mg/kg) did not reverse PCP- or amphetamine-induced locomotor activity in rodents, nor did it alter the effects of haloperidol (0.1 to 0.3 mg/kg) or olanzapine (0.03 to 3 mg/kg) in these tests. Interestingly, AVE1625 (10 and 3 mg/kg) decreased catalepsy induced by either haloperidol (1 mg/kg) or olanzapine (10 mg/kg). To determine the effects of AVE1625 on antipsychotic-induced weight gain, we treated rats for 14 days with either olanzapine or olanzapine plus AVE1625. While rats treated with olanzapine alone (3 mg/kg/day) gained significantly more weight than controls, rats treated with olanzapine (3 mg/kg/day) plus AVE1625 (10 mg/kg/day) did not gain more weight than controls.
Discussion: This profile makes AVE1625 an exciting candidate for further development as a treatment for cognitive deficits in schizophrenia.

101. Divergence of PPI Deficits and P50 Suppression Deficits in Schizophrenia Patients Point to Two Dissociable Endophenotypes: Implications for Genetic Analyses in the COGS Project
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Background: Schizophrenia is characterized by "core" cognitive disorganization, which is associated with impaired functional outcome. A major construct used to assess cognitive integration is "gating": the ability to automatically suppress responsivity to irrelevant stimuli and thereby enhance the cognitive impact of information-laden events. Two major paradigms have been used to assess gating deficits in schizophrenia patients: (1) prepulse inhibition (PPI) of the startle response, reflecting sensorimotor gating and (2) event-related P50 suppression, reflecting sensory gating. PPI and P50 suppression are both impaired in schizophrenia patients, their first degree relatives and in schizotypal patients. Both measures have animal homologs which have led to explication of their neural substrates. Yet despite their "face valid" similarities, it is unclear if both forms of gating deficits in schizophrenia patients are highly correlated. This possible convergence (or divergence) has important implications for the use of these measures as biomarkers in medication studies and as endophenotypes in studies of the genetic architecture of schizophrenia. This study of schizophrenia patients was designed to answer the question of the level of association of PPI and P50 suppression deficits in a large cohort of schizophrenia patients versus normal comparison subjects.
Methods: 84 schizophrenia patients and 64 carefully screened normal comparison subjects were all studied at the UCSD Medical Center: Demographic, symptom, medication and other variables were assessed; Diagnoses were obtained via SCID interviews; PPI was assessed via established methods, at 30, 60, 120 msec ISI's with a 115 dB startle stimuli and 85 dB prepulses over 70 dB background. P50's were assessed with 93 dB click pairs with a 500 msec ISI. A minimum of 120 artifact free click pairs were used to assess P50 suppression.
Results: ANOVA of PPI revealed a significant group x ISI interaction ($p < 0.001$); post-hoc comparisons revealed PPI deficits at the 60 msec ISI in patients ($p < 0.01$). For P50 suppression, ANOVA revealed patient deficits in P50 suppression ($p < 0.05$) and reduced S1 amplitude ($p < 0.05$), but normal S2 amplitude. The level of PPI (60 msec ISI's) and P50 suppression were not associated in schizophrenia patients (r's < .15). The influences of gender, symptom level and demographic variables are presented in the body of the poster.
Discussion: As predicted, a large cohort of schizophrenia patients (N=84) had deficits in both PPI (sensorimotor) and P50 event-related potential suppression (sensory) gating. Also, these two forms of gating deficits were not associated with each other in schizophrenia patients. PPI is subserved by a cortico-striato-pallido-thalamic (CSPT) circuitry that has interacting and overlapping neural substrates with P50 generation circuitry, especially in mesial temporal lobe structures. Still, these two forms of gating deficits are clearly dissociable in schizophrenia patients, where deficits in both of these measures are observed. The results of the present study suggest that PPI and P50 suppression are dissociable and complementary gating measures that can be used as informative and independent biomarkers in drug studies and as endophenotypes in studies of the genetic architecture of schizophrenia.

102. Elevated Prenatal Homocysteine as a Risk Factor for Schizophrenia
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Sponsor: Jean Endicott

Background: Context. Elevated prenatal homocysteine is a plausible risk factor for schizophrenia due to its partial antagonism of N-methyl-D-aspartate receptors at physiologic glycine levels and its association with abnormal placental function and pregnancy complications. We examined whether elevated maternal levels of homocysteine during the third trimester were associated with adult schizophrenia risk in a large birth cohort.
Methods: The cohort members were derived from the Child Health and Development Study (CHDS). During 1959-1966, the CHDS recruited virtually all pregnant women receiving obstetric care from the Kaiser Permanente Medical Care Plan (KPMCP) in Alamada County, California. Comprehensive data were prospectively collected from maternal medical records and interviews and maternal blood was drawn, and sera obtained, throughout pregnancy in nearly all subjects. The serum specimens were frozen immediately and archived at -20 degrees C in a single repository. Cohort members were followed up for schizophrenia from 1981 through 1997 by the registries of KPMCP, and cases (N=63) of schizophrenia spectrum disorders (83% with schizophrenia or schizoaffective disorder) were diagnosed by face-to-face assessment with the Diagnostic Interview for Genetic Studies. Controls (N=122) belonged to the birth cohort, had not been diagnosed with a schizophrenia spectrum or major affective disorder, and were matched to cases on date of birth, gender, length of time in the cohort, and availability of maternal sera. Archived maternal sera were assayed for homocysteine levels during pregnancies of cases and matched controls using high performance liquid chromatography with coulometric electrochemical detection.
Results: In a model which tested for a threshold effect of third trimester homocysteine levels, elevated homocysteine was associated with a greater than twofold, statistically significant increase in schizophrenia risk in the offspring (OR=2.39, 95% CI=1.18, 4.81, p=.015). There was no effect of first trimester homocysteine (OR=1.91, 95% CI=0.52, 7.06, p=0.33) or second trimester homocysteine (OR=0.49, 95% CI=0.19, 1.31, p=0.16) on risk of schizophrenia. There was no evidence of a graded effect of homocysteine during any trimester on risk of schizophrenia.
Discussion: These findings suggest that hyperhomocysteinemia during the third trimester of pregnancy may be a risk factor for schizophrenia. Homocysteine is a partial NMDA receptor antagonist at physiologic glycine concentrations, and perinatal NMDA receptor antagonism appears to have long-lasting effects on locomotor response, working memory, and pre-pulse inhibition that are analogous to abnormalities observed in schizophrenia. Hyperhomocysteinemia also disrupts placental function by activation of coagulation factors, induction of endothelial damage, and apoptosis of trophoblasts; the resulting placental pathology may act to increase risk of schizophrenia by compromising delivery of oxygen and nutrients to the developing fetus. Deficiencies of folate and vitamin B12, and the C677T polymorphism in the gene for methylenetetrahydrofolate reductase, are well-known causes of elevated homocysteine, and may underlie the observed effects. Although replication is required, the results suggest that supplementation with folic acid, or other vitamins

which reduce homocysteine levels, may be considered as a potential future strategy to facilitate a reduction in the risk of schizophrenia.

103. Evidence That a DTNBP1 Risk Haplotype is Associated with Negative Symptoms and Cognitive Dysfunction in Patients with Schizophrenia

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Sponsor: Terry Goldberg

Background: Several schizophrenia susceptibility genes have been recently identified including catechol-o-methyltransferase, neuregulin 1, disrupted in schizophrenia, and dysbindin (DTNBP1) (Harrison & Weinberger 2005). However, schizophrenia is a clinical syndrome with considerable variation in the biological correlates, symptomatology, and functional outcome within this larger diagnostic category. Information regarding the impact of these genes on specific phenotypic traits (i.e. negative symptoms, cognition, delusions) is important in facilitating our understanding of the underlying pathology of schizophrenia. DTNBP1 is perhaps the strongest candidate gene for schizophrenia (Owen et al. 2005). Although its specific mechanism of action is not known, preliminary data suggest that DTNBP1 genotype mediates the risk for schizophrenia through reduced DTNBP1 expression (Bray et al. 2005) and associated decreased glutamatergic release (Numakawa et al. 2004). As glutamatergic antagonists produce negative symptoms and cognitive impairment in healthy individuals and exacerbate negative symptoms and cognitive impairment in patients with schizophrenia (Malhotra et al. 1997), DTNBP1 may be acting to increase risk for specific glutamate-based symptom domains.
Methods: We have therefore completed a series of studies to test the hypothesis that DTNBP1 genetic variation would be associated with schizophrenia, negative symptoms, and cognition.
Results: In Study 1, comprised of 524 patients with schizophrenia or schizoaffective disorder and 573 healthy volunteers, we genotyped seven single nucleotide polymorphisms within the DTNBP1 gene and identified a 6-locus haplotype (CTCTAC) that was significantly over-represented in the Caucasian patients compared to Caucasian healthy volunteers (p=0.005). The minor alleles of three individual SNPs [P1578-(rs1018381), P1763-(rs2619522), and P1765-(rs2691528)] were also significantly over-represented in patients (Funke et al. 2004). Study 2 included a subset of 181 patients with schizophrenia for whom comprehensive clinical data was available. Structured Clinical Interview DSM-IV (SCID) ratings were used to assess for lifetime history of negative symptoms. We detected a significant relationship between the CTCTAC haplotype and a history of prominent negative symptoms using the combined negative symptom rating (p=0.01) and for each individual item [avolition (p=.04), alogia (p=.01) and flattened affect (p=.016)]. Finally, in Study 3 we examined DTNBP1 genotype in a subset of patients with schizophrenia (n=213) and in healthy volunteers (n=137) who were characterized for their cognitive performance. We evaluated the relationship between the CTCTAC haplotype and a measure of g (the 1st unrotated factor of a PCA with a number of diverse cognitive measures), as well as estimated IQ (WRAT-3 Reading) and found that haplotype carriers had lower g (p=0.03) and lower estimated IQ (p= 0.03) as compared with non-carriers. Furthermore, when we characterized the patient sample as cognitive deficit patients versus cognitively spared patients, we found that the cognitive deficit patients were more than twice as likely to carry the risk haplotype (33%) than the cognitively spared patients (15%) (p=0.01).
Discussion: These data suggest that DTNBP1 may impact upon key symptom domains of schizophrenia, such as negative symptoms and impaired cognition. This represents an initial step toward the molecular classification of illness.

104. Hypometabolic Medial-Frontal Effects of Chronic Intermitent PCP Exposure in Rats Assessed with High Resolution Magic Angle Spin 11.7T Proton Magnetic Resonance Spectroscopy

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Sponsor: Jan Fawcett

Background: The NMDA hypofunction model of schizophrenia proposes that NMDA dysfunction of inhibitory neurons leads to a downstream unrestrained chronic hyperglutamatergic excitotoxic state, which results in neuronal damage in widely distributed cortical brain areas and may account for the poor clinical outcomes (Olney, 1995). We tested the effect of chronic NMDA hypofunction in rats, on proton-MR spectroscopy measures, like NAA, glutamine, glutamate and GABA concentrations in various brain regions.
Methods: Phencyclidine (PCP), 2.58 mg/kg/day, IP, a total of 14 daily injections was given intermittently over a 26 day period as described by Cochran et al (2003)to 10 male adult Sprague-Dawley rats. Ten animals received saline. Brains were removed and the following 2mm circular punches obtained: medial prefrontal, cingulate, retrosplenial and auditory cortices, striatum, accumbens, dorsal and ventral hippocampus and amygdala. HR-MAS 1H-MRS analysis was performed on the tissue punches using a Bruker Avance 11.7T 500 MHz magnet. Spectra were analyzed with a custom LCModel (Provencher, 1993).
Results: For NAA, the main metabolite of interest, there was a drug by region interaction ($F(5,18)=4.3$;$p=0.009$) with lower mediofrontal NAA in the PCP group. In this region there were also significant PCP related reductions for aspartate, creatine, GABA, glycine, glutamate, inositol, lactate, PEA and taurine ($p<0.05$).
Discussion: A low dose one month exposure of PCP in adults rats results in reductions in many neurometabolites visible in the proton spectrum consistent with clinical findings in schizophrenia. The medial frontal cortex is particularly affected. This chronic PCP model may prove useful in suggesting specific neurometabolic abnormalities to be studied in clinical populations.

105. The Eyes Don't Have It: Face Processing in Schizophrenia

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Sponsor: Daniel Javitt

Background: While a number of studies show that patients with schizophrenia have deficits in face emotion recognition, it is less clear whether they have deficits associated with general face processing mechanisms. Recent evidence examining the face inversion effect, or increased ability to recognize upright as opposed to upside down faces, has shown that a similar inversion effect is found whether parts of the face or spacing between parts has been altered, with no inversion effect for houses. This suggests that the same mechanism is used for processing part and spacing information. Thus, the face inversion effect may reflect holistic processing engaged by faces such that faces are represented as wholes and not decomposed into parts. There is also electrophysiological evidence in controls for an inversion effect that is greater for faces than objects. To further explore whether patients have a deficit in face processing and what mechanism(s) are utilized by patients, we examined upright versus inverted faces and objects along with an electrophysiological paradigm looking at the N170 to faces and objects.
Methods: In experiment 1, patients with schizophrenia and controls were asked to determine whether pairs of upright or inverted faces or houses that differed in either the spatial distance among parts (configuration) or the shape of the parts (part) were the same or different.

In order to be able to detect an inversion effect, if present, patients were given enough time to view the pictures to obtain a score of 70% correct on upright houses. In experiment 2, high density electrophysiological recordings were obtained to pictures of upright and inverted faces, along with objects (watches, flowers) of similar general configuration and spatial frequency content.
Results: In Experiment 1, the inversion effect was significant for faces but not for houses as confirmed by a highly significant interaction ($p<0.001$) between Orientation (upright/inverted) and Stimulus (house/face). Importantly, the inversion effect was comparable across patients and controls as indicated by a non-significant interaction between Orientation, Stimulus and Group ($p=0.14$). Experiment 2 showed that the inversion potential (N170 modulation) obtained by subtracting the upright from inverted faces was similar between patients and controls.
Discussion: Results of behavioral and electrophysiological studies provide strong evidence that face processing is normal in patients with schizophrenia. Further, the behavioral experiment included part as well as spacing tasks for faces and houses. This sheds light on mechanisms involved in face processing. The recent finding of a similar face inversion effect for part and spacing tasks suggests a holistic processing mechanism in normal controls. The results with patients suggest that they use the same holistic processing mechanism as controls.

106. Neurocognitive Deficits in Prodromal and First Episode Schizophrenia are Stable and Predictive of Social Functioning

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Sponsor: David Braff

Background: Schizophrenia is a devastating illness that emerges during a crucial period of neurodevelopment. Early identification and characterization of individuals in the prodromal phase and first episode of schizophrenia using vulnerability markers for psychosis may add important insight into neurodevelopmental processes and predict evolution of psychosis and functional outcome. Vulnerability markers, including a neurocognitive battery, were selected because of established deficits across schizophrenia spectrum groups (schizophrenia , unaffected first degree relatives and schizotypal personality disorder), high reliability in repeated testing and evidence of heritability.
Methods: Subjects at risk for schizophrenia (AR N=37), based on family history of schizophrenia plus a decline in functioning or the new onset of subsyndromal psychotic symptoms, were compared to first episode schizophrenia patients (FE N=15) and normal comparison subjects (NC N=37) at baseline then at 6 and 12 month follow-up. The neurocognitive battery included the domains of executive functioning, verbal memory, processing speed, working memory and general intelligence. The stability of neurocognitive performance and practice effects over repeated testing were then analyzed. Social functioning was assessed using the Social Adjustment Scale at 1 year. Baseline neurocognitive performance was then used to predict social functioning at follow-up in the AR sample.
Results: There were no significant differences between groups in age, parental education, gender or handedness. At baseline assessment, AR and FE subjects demonstrated deficits across multiple cognitive domains (overall impairment index: $F(2,88) = 17.53$, $p<.0001$; MANOVA $F[10,166]=3.93$, $p<0.001$, unadjusted effects across all five neurocognitive domains $p<0.05$) compared to NC subjects. Post-hoc analyses indicated that the AR subjects significantly differed from the NC across all domains and were intermediate to the NC subjects (effect size = 0.70, $p<0.001$) and the FE sample ($p<0.05$) on the Overall Impairment Index. In repeat testing, all tests were stable with moderate to good test-retest correlations (r=.43-.95) between baseline (T1) and 6 months (T2) in 68 subjects (NC=19, AR=36, FE=13). Significant differences between performance at T1 and T2, reflecting practice effects, were noted across a number of measures but effect sizes were in the small to moderate range. These data were also analyzed using the Reliable Change Index (RCI) Method to adjust for practice effects and estimate the expected range of performance on each measure based on a 90% confidence interval. Few subjects fell outside the expected distribution on any of the tests. Regression analyses were performed to determine whether performance on neurocognitive domains was predictive of later social functioning. Although a number of the neurocognitive domains at baseline were significantly correlated with social functioning at follow-up, general intelligence and processing speed variables provided the best model fit, accounting for 40% of the variance in social functioning ($F[2,9]=4.65$, $P<0.05$).
Discussion: Neurocognitive deficits are evident in the first episode of schizophrenia and may be present in the prodromal phase of the illness. The observed deficits are stable with repeated testing and have utility as endophenotypic markers in future genetic analyses similar to the Consortium on the Genetics of Schizophrenia (COGS). Longitudinal follow-up will allow further assessment of outcome measures such as social functioning and psychotic conversion in AR subjects and the relationship of baseline neurocognitive functioning to outcome.

107. The Consortium on the Genetics of Schizophrenia (COGS): Recruitment and Assessment Methods in a Multi-Site Collaboration

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Sponsor: Bruce Turetsky

Background: The COGS is an ongoing NIMH funded 7-site collaboration investigating the genetics of schizophrenia endophenotypes. Multisite collaborations require careful orchestration and calibration of methods across sites. The purpose of the current report is to provide a detailed description of COGS participant recruitment and assessment efforts.
Methods: All participants are between the ages of 18-65, able to understand consent and participate in testing of >=1 endophenotype. Schizophrenia probands are recruited from clinics/clinician referrals, local NAMI chapters, and the media. Eligible probands are medically healthy and have both biological parents available for genotyping and >=1 unaffected full sibling available for endophenotyping and genotyping. Probands having only 1 available parent but 2 or more available siblings are also included though this configuration is less powerful for genetic analyses. Proband exclusion criteria are: both parents or all siblings diagnosed with schizophrenia, recent ECT, positive illicit drug screen, recent substance related disorder, premorbid IQ<70, and psychiatric stability <1 month. First-degree biological relatives of probands are excluded from endophenotype testing if they are intoxicated at the time of testing or have severe systemic illness. Controls are recruited primarily through advertisements (newspapers, local flyers and websites) and included if they are medically healthy and have no history of Cluster A personality disorders or personal or family history of psychosis. To parallel comorbidity in relatives of probands, other non-psychotic Axis I psychopathology is accepted, but clinical stability and/or remission is required. All participants undergo a standardized assessment protocol including the Diagnostic Interview for Genetic Studies (DIGS), Family Interview for Genetic Studies (FIGS) and medical record review. Interviewers are trained using a standardized procedure. Each participant is assigned a DSM-IV best estimate final diagnosis (BEFD) based on consensus review by >= two faculty level clinicians. A pilot study of inter-site reliability of BEFD for five cases yielded 100% agreement for the BEFD of schizophrenia. Following diagnostic assessment, eligible participants are categorized as "broad" or "narrow" based on presence (broad) or absence (narrow) of significant comorbid lifetime medical or psychiatric illness. This characterization enables subset analyses restricted to medically and psychiatrically healthy participants. A committee of investigators and clinical personnel participate in fortnightly teleconferences to coordinate and evaluate recruitment and assessment progress and procedures.

Results: As of July 2005, 515 participants were endophenotyped, including schizophrenia probands (n=108, M:F=78:30), relatives (n=267, M:F =112:155) and controls (n=140, M:F=63:7). Probands (mean age=34.3 yrs,SD=10.8) and controls (M=36.8,SD=12.4) are significantly younger than relatives (M=45.6,SD=14.2; p's<.05), though the subset of siblings (n=161; M=37.0,SD=11.8) does not differ from controls. As can be expected, probands (mean ed=13.6 yrs,SD=2.1) have less education than controls (M=15.4,SD=2.9) and relatives (M=15.5,SD=2.8; p's<.05). More than half of participants fall in the narrow category (probands=62.7%; relatives=57.7%, controls=68.8%). Site differences will be explored.
Discussion: Multi-site genetics collaborations require assessment and recruitment methods that are clearly defined, well communicated and uniformly applied. We have presented methods and preliminary demographics of a sample for whom the genetics of endophenotype performance will be fully characterized.

108. Prefrontal Cortex Inefficiency During Working Memory in Unaffected Siblings of Patients with Schizophrenia: A Replication
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Sponsor: Daniel Weinberger

Background: Exaggerated prefrontal cortical (PFC) response in working memory (WM) tasks previously reported in unaffected siblings of schizophrenia patients, mirroring that in patients when they performed close to normal, implicate physiological abnormalities in the PFC as possible intermediate endophenotypes in the search for disease susceptibility genes (Callicott 2003). However, it has also been recently found that patients with schizophrenia also increase activation to a larger extent in ventral PFC in response to increasing working memory load (Tan et al., in submission). It has been argued that this ventral PFC activation is in compensation for the combination of PFC dysfunction in the setting of poorer WM function. Thus, we sought to further explore the familial aspects of PFC function in schizophrenia by looking at the effect of varying WM load in unaffected siblings. Specifically, since unaffected siblings perform as well as healthy volunteers on our WM task, we hypothesized that we would find dorsal, but not ventral PFC abnormalities in siblings.
Methods: We studied 16 pairs of matched normal volunteers and unaffected siblings of schizophrenia patients as they underwent 3T fMRI while performing 1 and 2-back WM tasks.
Results: Siblings and controls did not differ in N-back accuracy or reaction time. However, PFC activation was increased in unaffected siblings relative to controls within the dorsal PFC, where there was a main effect of group, WM load, and a load-by-group interaction. This was driven by increased activation at the 2-back in siblings. Ventral PFC activation showed a main effect of WM load but no group or load-by-group interaction.
Discussion: We have replicated our earlier findings that unaffected siblings of patients with schizophrenia have inefficient PFC function during WM challenge, but specifically only at higher WM load. As predicted, siblings did not show abnormal ventral PFC function and this may support arguments that dorsal PFC function in more primary to the illness or at least more likely to be heritable.

109. Promoter Specific Alterations of BDNF mRNA in Frontal Cortex in Patients with Schizophrenia
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Sponsor: Jacqueline Crawley

Background: BDNF is an important regulator of cortical neuronal maturation and signaling. Regulation of BDNF mRNA synthesis is complex and includes transcription start sites from multiple 5' promoters that are differentially regulated in development and by antidepressant drug exposure. Previously, we and others have found a significant reduction in pan BDNF mRNA in the frontal cortex of patients with schizophrenia. However, it is not known from which alternative promoter this BDNF mRNA reduction derives.
Methods: Using quantitative PCR we analyzed frontal cortical cDNA from schizophrenic patients (n=35) and normals (n=35) matched for gender, age, pH, and PMI. First, we determined that there were no group differences between four housekeeping mRNA species (GUSB, SDHA, Cyclophilin, and PBGD) in this sample. We measured three specific BDNF transcripts (1-5, 2-5, 3-5) using the ^^Ct method and normalizing to the geometric mean of the four control mRNAs.
Results: Unexpectedly, we detected a two-fold increase in BDNF 1-5 mRNA in schizophrenic patients compared to normals (F=4.43, df=1,53, p=0.039) using a two way ANCOVA with diagnosis and gender as independent factors and covarying for age and pH. In contrast, there was no significant diagnostic effect for BDNF 2-5 or 3-5 mRNA (all p>0.05). However, a main effect of gender was detected in BDNF 2-5 mRNA (F=4.40, df=1,46, p=0.041). When comparing male patients with schizophrenia to normal males we found a 35% decrease in BDNF 2-5 mRNA (p=0.019). No other gender differences are found in either BDNF 1-5 or 3-5 transcripts (all p>0.05). Next, we asked if antidepressant medication impacts BDNF mRNA levels in patients. We found a significant two-fold increase in BDNF 2-5 and 3-5 mRNA (t>-0.46, p<0.021) in patients with a history of antidepressant medication based on toxicology reports and/or clinical records.
Discussion: Our data suggests that frontal cortical BDNF mRNA could be influenced by diagnosis, gender, and treatment. However, these influences are differential and may act in particular 5' regions of the BDNF gene. Our results also suggest that further analysis of additional BDNF promoters is necessary to determine the source of the overall reduction in pan BDNF mRNA.

110. Trait vs. State Markers for Schizophrenia: the Case of Visual Motion Processing
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Background: Identification and characterization of behavioral markers that are intrinsic to complex mental disorders like schizophrenia are central for neuropsychopharmacology. Trait markers index the behavioral and biological processes that play an antecedent, possibly causal, role in the pathophysiology of the disposition to schizophrenia, whereas state markers reflect the status of clinical manifestations in patients. Psychosis, the hallmark of schizophrenia, alone is unable to serve to differentiate the two types of markers. Certain sensory and cognitive processes, identified and characterized through basic science research, show promise as trait markers for schizophrenia. The differentiation of types of visual functions that can serve as trait or state markers is beginning to receive attention. Examining individuals who do not manifest any psychotic symptom and who are biological relatives of patients can provide information on the roles of the disposition to schizophrenia in visual responses. Comparisons with bipolar patients who also manifest psychotic symptoms can provide insight about the specificity of visual dysfunction to schizophrenia.
Methods: We examined visual motion integration and visual motion discrimination in schizophrenia patients, their biological relatives, bipolar patients and normal controls. Using psychophysical methods, we measured thresholds in detection of coherent motion and velocity discrimination. Detection of coherent motion requires integration of spatially distributed motion signals whereas velocity discrimination requires differentiation of motion signals at the same spatial locations.
Results: The thresholds of detecting coherent motion were significantly elevated (impaired) in schizophrenia patients but not in their

relatives and bipolar patients. The thresholds of velocity discrimination were significantly elevated in schizophrenia patients and their relatives but not in bipolar patients.
Discussion: The pattern of the visual dysfunctions suggests that motion discrimination possesses trait characteristic of schizophrenia and motion integration does not.

111. Identification and Characterization of Behavioral Plasticity Genes in *Drosophila*

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Plasticity in the nervous system allows an organism to learn about its environment, remember its experiences, and appropriately alter its behavior. Understanding how neurons accomplish this plasticity is fundamental to understanding normal, and perhaps pathological, brain function. It is conceivable that complex psychiatric disorders such as depression, anxiety, thought disorders, and addiction, arise partly from imperfect adaptation of the brain to its environment. Certainly, psychiatric treatments, including various psychotherapies and pharmaceuticals, exploit the brain's ability to change. However, the molecular study of plasticity in complex human behavior is difficult. Thus, neuroscientists have turned to model systems.
Methods: Habituation is a simple form of behavioral plasticity and nonassociative learning that is defective in some schizophrenics. We recently developed a computerized assay for tracking fly movements. Using this assay, we conducted a forward-genetic screen in the fruitfly, *Drosophila melanogaster*, to identify genes that affect habituation of a startle response. We generated 2000 lines by random P-element mutagenesis and screened approximately 1000 for defects in startle habituation.
Results: We identified several genes that affect habituation behavior. Selected genes that, when mutated, produce enhanced habituation include *Protein tyrosine phosphatase 10D (Ptp10D), Gliotactin (Gli), Inositol 1,4,5-triphosphate kinase 1 (IP3K1)*, and *14-3-3* ζ. In contrast, a mutation in *shaggy*, the *Drosophila GSK3*β homolog, reduces habituation. We have begun to characterize *Ptp10D*, a receptor protein tyrosine phosphatase that has not previously been shown to affect behavior.
Discussion: The results of this screen may lead to novel mechanisms involved in behavioral plasticity, and possibly, in some types of psychopathology.

112. Confirmation of the Association of 10 Genes with Atypical Anti-Psychotic Treatment Emergent Weight Gain in a Cohort with an Obesity Phenotype

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Sponsor: Alan Breier

Background: Treatment emergent weight gain continues to be a significant challenge for atypical anti-psychotic therapy, as predicting those individuals most susceptible for gaining weight following induction of anti-psychotic treatment remains unclear. Although the mechanism underlying treatment emergent weight gain remains largely unknown, genetic influence has been proposed. We employed a genome wide association study to uncover potential genetic contributions to treatment emergent weight gain. Using Perlegen Sciences Inc. genotyping platform, greater than 1.4 million single nucleotide polymorphisms (SNPs) were analyzed for their relationship with weight gain in a cohort of patients who received olanzapine for a minimum of 6 months. Using Fishers exact, 311 SNPs were significantly associated with treatment emergent weight gain at the $p<0.001$ level, and >600 SNPs at the $p<0.005$ level.
Methods: In order to investigate which of these SNPs were also associated with an obese phenotype, a cohort of 348 parent-child trios was analyzed. The trios were selected for obesity with the criteria of the child having a BMI greater than 35 kg/ m2. SNPs significantly associated with weight gain from the genome wide association analyses (n=668 SNPs) were supplemented by additional SNPs in linkage disequilibrium (n=3073) with these SNPs for a total of 3741 SNPs. The obesity trios were genotyped and association of the SNPs with BMI was completed using the quantitative transmission disequilibrium test.
Results: With a significance criteria of $p < 0.01$, 103 SNPs were associated with BMI. These SNPs were not randomly distributed across genes but rather clustered in several genes. Genes that were significant in the genome wide association study for treatment emergent weight gain and that also confirmed in the obesity cohort include A2BP1, ADARB2, EFA6R, EPB41L4A, EPHA7, PCAF, PKHD1, ROS1, SLC8A1, and TOX.
Discussion: Genetic and haplotype association and interesting biological correlates for these genes with other data such as that from mRNA microarray and in situ hybridization will be discussed.

113. Patient-Reported and Clinician-Rated Depressive Symptoms in the Treatment of Schizophrenia

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Background: To assess the concordance between patient-reported and clinician-rated depressive symptoms in the treatment of people with schizophrenia, and identify which functional outcomes are more strongly linked to clinician-rated compared to patient-reported depressive symptoms.
Methods: We used data from a large prospective naturalistic multi-site 3-year study of treatment for people with schizophrenia in the United States, conducted between 7/1997 and 9/2003 (US-SCAP). Depressive symptoms were assessed with the 10-item clinician-rated Montgomery-Asberg Depression Rating Scale (MADRS) at enrollment and at 12-month intervals thereafter. Patient-reported depressive symptoms were assessed with 4 items from the SCAP-Health Questionnaire (SCAP-HQ), a validated self-report measure administered at enrollment and at 6-month intervals thereafter. The patient-reported depressive items were rated for the prior 4 weeks on a scale from 1 (not at all) to 5 (extreme), assessing: feeling unhappy or sad; low energy level; hopelessness about the future; and feelings of worthlessness. Patients answered "yes/no" to suicidal thoughts or suicide attempts. Functional outcomes included use of mental health services, safety to self and others, substance use, productivity, activities and relationships, and quality of life. Functional outcomes were assessed with self-report measures (e.g., the Medical Outcomes Study Short Form 12, CAGE, SCAP-HQ), clinicians' ratings (Quality of Life Scale), and systematic abstraction of medical record information. Pearson Product-moment correlations calculated the associations between patient (SCAP-HQ) and clinician-rated (MADRS) depressive symptoms, and between two clinician-rated measures of depressive symptoms at enrollment (MADRS and PANSS depression subscale). Mixed model and General Estimating Equations were used to estimate whether each outcome measure was more robustly linked to patient-reported or to clinicians' ratings of depressive symptoms across the 3-year study period.
Results: At enrollment, participants (N=2,228) were moderately ill (mean PANSS total score: 70.2), and were primarily (94.6%) outpatients with average illness duration of 20.2 years. Patient-reported and clinician-rated depressive symptoms were moderately and significantly correlated ($r=.65$, $p<.001$). The correlation between the two

clinician-rated measures of depressive symptoms was r=.77 (p<.001). Clinician-rated depressive symptoms were more strongly associated with specific functional outcomes (e.g., greater likelihood of substance use, of violent behavior, of emergency room use, lower GAF scores, and poorer medication adherence), whereas patient-reported depressive symptoms were more strongly associated with other functional outcomes (e.g., level of daily activity, leisure activity, family relations, and general life satisfaction). Patient-reported and clinician-rated measures of depressive symptoms were similarly associated with suicidal ideation and suicide attempt.
Discussion: Moderate and significant concordance between patient and clinician ratings of depressive symptoms suggests that people with schizophrenia can reliably rate their depressive symptomatology. Furthermore, patient-reported depressive symptoms appear to be more strongly linked to different functional outcomes than clinician-ratings. These findings demonstrate a consistent and robust link between depressive symptomatology and functional outcomes, and highlight the importance of monitoring depressive symptoms in people with schizophrenia, using both patient and clinician-rated measures.

114. Investigating Cortical Inhibition as a Potential Mechanism of Atypicality in the Antipsychotic Clozapine Using Transcranial Magnetic Stimulation

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: It has been previously demonstrated that unmedicated patients with schizophrenia have deficits in cortical inhibition (CI) as indexed with transcranial magnetic stimulation (TMS). This inhibition is largely mediated by cortical GABAergic mechanisms. These inhibitory deficits may be attenuated with the use of antipsychotic medications, particularly atypical antipsychotic medications. Also, animal studies suggest that clozapine enhances GABAergic inhibitory neurotransmission. The purpose of this study, therefore, was to examine the effects of clozapine on TMS measures of CI and to compare these effects to patients treated with other antipsychotic medications and healthy controls.
Methods: We used two TMS inhibitory paradigms: short interval intracortical inhibition (SICI) and cortical silent period (CSP) to evaluate CI in 10 clozapine treated patients with schizophrenia, 6 unmedicated patients with schizophrenia and 10 healthy control subjects.
Results: Clozapine treated patients with schizophrenia had significantly longer CSPs compared to healthy controls (p=0.02) trended towards longer CSPs compared to unmedicated patients with schizophrenia (p=0.06). There was no significant difference between groups as indexed with SICI.
Discussion: Clozapine treated subjects have significantly prolonged CSP compared to healthy controls and unmedicated patients with schizophrenia. As the CSP may be related to GABAB neurotransmission these findings suggest that clozapine is a facilitator of this neurotransmitter system that may account for some its atypical properties.

115. Null Mutation of the Alpha-2A Adrenoceptor in Mice Fails to Alter Visuospatial Attention or the Performance Enhancing Effects of Guanfacine

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Sponsor: Robert Roth

Background: Previous work has demonstrated that alpha-2 adrenoceptor agonists markedly attenuate the cognitive deficits produced by NMDA receptor hypofunction. Briefly, clonidine and guanfacine dramatically increase both working memory and attentional performance in rodents treated with PCP. Our current interests involve exploring the adrenoceptor subtype involved in these pharmacological effects.
Methods: Adult male alpha-2A subtype null mutant mice (either heterozygous or homozygous for the mutant allele) were used. This line is pure C57Bl/6J background. Genotypes were confirmed by PCR. All mice were first mildly food restricted (to 80-90% of free feeding weights) and then trained to perform a lateralized reaction time test in operant conditioning chambers. After determining baseline performance, the mice were challenged with combinations of either saline or guanfacine (0.05-1.0 mg/kg, s.c.) and saline or PCP (1.0 mg/kg, s.c.). All mice received all pharmacological treatments in a randomized, Latin Squares design.
Results: Both acquisition and performance of the lateralized reaction time test was assessed for main effects of genotype to determine whether alpha-2A null mutation affected attention. After examining 29 mice (n=16 het and n=13 hom), no main effects of genotype were detected, meaning that the alpha-2A receptor is not critical for optimal attentional performance. Moreover, PCP impaired performance in both +/- and -/- mice, with a tendency for the deficit to be larger in -/- mice. Finally, guanfacine enhanced attentional performance in both genotypic groups after PCP treatment.
Discussion: These data indicate that the alpha-2A adrenoceptor subtype is not esssential for attentional performance in mice, nor is it critical for the performance enhancing effects of guanfacine in this assay. Further studies will be required to determine whether null mutation of the alpha-2B and -2C subtypes produce similar or dissociable effects.

116. Ziprasidone Dosing Study in Pediatric Patients with Bipolar Disorder, Schizophrenia, or Schizoaffective Disorder

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Background: Children and adolescents with mood and psychotic disorders are often treated with atypical antipsychotics. However, few studies have evaluated the dosing and tolerability of ziprasidone in this population. We compared the safety, tolerability, and effectiveness of 2 dose-titration schedules of ziprasidone in youth with bipolar mania, schizophrenia, or schizoaffective disorder.
Methods: Subjects were randomized to ziprasidone monotherapy titrated over 7 to 10 days from 10-40 mg BID (Group 1) or from 20-80 mg BID (Group 2). Both groups were then treated at fixed doses for up to 3 weeks (Period 1). Subjects could continue flexible-dose treatment for 6 months (Period 2), during which concomitant therapy was permitted. Inclusion criteria included ages 10 to 17 years; bipolar I disorder (manic or mixed) and a YMRS score >=17; or schizophrenia/schizoaffective disorder and a BPRS score >=35 and a score of >4 on at least one of unusual thought content, hallucinations, suspiciousness, or conceptual disorganization. Study visits were on Day 4 and Weeks 1, 2, 3, 4, 8, 12, 18, and 27. Safety and tolerability measures included serum laboratory measures, body weight and height, ratings of extrapyramidal symptoms, vital signs, ECGs, as well as spontaneously reported adverse events (AEs). Exploratory efficacy measures were YMRS (bipolar subjects) and BPRS-A, CGI-S, and CGI-I (all subjects).
Results: Twenty-three subjects were enrolled into Group 1 (15 bipolar, 8 schizophrenic/schizoaffective). Forty subjects were enrolled into Group 2 (31 bipolar, 9 schizophrenic/schizoaffective). Thirty-one of the 56 (89%) subjects who entered into Period 2 completed the entire 6 months. No unexpected safety or tolerability findings occurred. During Period 1, the most common AEs in Groups 1 and 2 were sedation (21.7%, 35.0%), somnolence (34.8%, 27.5%), nausea (21.7%, 27.5%), headache (13.0%, 27.5%), dizziness (13.0%, 22.5%), and vomiting (13.0%, 20%). During Period 1 ziprasidone doses were reduced or discontinued due to AEs in 7/23 subjects (30%) in Group 1

vs 22/40 subjects (55%) in Group 2. Mean (SD) QTc (Fridericia) change (around Cmax) from baseline to week 3 was 3.6 (20.6) msec in Group 1 and 10.0 (16.3) msec (range, 23.5 to 46.0 msec) in Group 2. No confirmed QTc (Fridericia) intervals > 500 msec occurred. During Period 2, only sedation (28.6%) and somnolence (30.3%) occurred in >20% of subjects. No clinically relevant changes in lipid profile or fasting glucose were observed. Weight gain was reported as an AE in 8.9%. Serious AEs (n = 19) were reported in 16 subjects, none related to treatment, including 5 cases of suicidal ideation and 1 of self-harm. The presence of suicidal ideation before the study, psychosocial stressors and the underlying disorder explained each of these events. Mean (SD) reductions from baseline to week 27 in CGI-S scores in bipolar subjects were 1.47 (1.51) for Group 1 and 1.33 (1.24) for Group 2 and in schizophrenic/ schizoaffective subjects and were 1.50 (1.20) in Group 1 and 2.14 (0.69) in Group 2. At week 3, mean (SD) YMRS improvement was 14.9 (9.7) in Group 1 and 11.1 (9.3) in Group 2 within the bipolar subjects. Mean (SD) BPRS score reductions were 9.0 (9.3) in Group 1 and 14.0 (6.7) in Group 2 in the schizophrenic/schizoaffective subjects.
Discussion: These results suggest that ziprasidone is well tolerated and may be effective in children and adolescents with bipolar mania, schizophrenia, or schizoaffective disorder. The results also indicate that a starting dose of ziprasidone 20 mg/day, titrated to a target range of 120-160 mg/day (60-80 mg BID) achieved over 1 to 2 weeks, may be optimal for most patients. Further evaluation of ziprasidone in controlled trials in pediatric populations is needed.

117. Brain Structural and Functional Deficits in Subjects at High Genetic Risk for Schizophrenia

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Background: Patients with chronic schizophrenia are known to have brain structural and functional deficits, particularly in frontal and temporal cortex and their connections. We currently are examining individuals with schizophrenia and their unaffected siblings who are within the age range of risk for developing illness. We aim to determine brain imaging predictors of who is most likely to develop schizophrenia on follow-up.
Methods: Siblings diagnosed with and without schizophrenia have been studied (ages 12-30). Scans were acquired on a 1.5T Siemens Vision System. 3D T1-weighted sagittal MPRAGE, axial Magnetization Transfer (MT), and Diffusion Tensor Images (DTI), and T2 images were completed. A f-MRI paradigm using a word discrimination task was developed to examine language processing. Data were analyzed using previosuly developed in-house and published software and compared across groups.
Results: 80 subjects were scanned. Thus far, a subgroup of these have been analyzed (15 high-risk, 15 with schizophrenia and 15 controls). Of these, the MT Ratio (MTR) was reduced in high-risk subjects compared with controls in the left superior temporal cortex, bilateral superior frontal and cingulate gyri. The Fractional Anisotropy (FA) as calculated from DTI, a measure of white matter integrity, was reduced in the left superior temporal gyrus, left cerebellum and precuneus. There were no structural volume differences between high-risk individuals and controls. Those with schizophrenia had increased ventricular volumes compared with both high-risk siblings and controls. fMRI activation in response to the language paradigm did not detect differences in the high-risk subjects in temporal or frontal cortex, but did show significantly different occipital lobe activation from controls. Their family members with schizophrenia, however, had more wide spread activation differences—ie in superior temporal and frontal regions when comared with controls.
Discussion: Thus far, these initial data suggest that some brain imaging anomalies may develop after the onset of illnes, but others may be early signs that illness is likely to occur. The exporatory occipital lobe findings in individuals at high-risk for schizophrenia are interesting in light of recent data from other groups suggesting early visual processing deficits in schizophrenia and thus difficulties in reading. This study will be expanded with a larger N and more detailed analyses.

118. Population Pharmacokinetic Analysis of Drug-Drug Interactions Among Risperidone, Bupropion, and Sertraline in CF-1 Mice

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Background: Atypical antipsychotics (APD) and antidepressants are increasingly co-prescribed. Some evidence exists for drug-drug interactions at the level of molecular targets. While the consequences of modulatiing centrally-mediated effects from combining drugs from these classes are unclear, equally uncertain is if and how such drug combinations interact to influence one another's metabolic disposition. Risperidone (RISP) which is metabolized by cytochrome P450 (CYP) 2D6 and transported across the blood brain barrier (BBB) by P-glycoprotein (P-gp) was studied in combination with bupropion (BUP), a potent CYP2D6 inhibitor with negligible P-gp effects, and with sertraline (SERT), a weak inhibitor of CYP2D6 but a potent P-gp inhibitor.
Methods: RISP, BUP and SERT were administered intraperitoneally into CF1 mice at doses of 100, 10, and 1 mcg/g mouse, respectively. Plasma and brain samples were collected at timed intervals from 0.5 to 6 hours. A pharmacokinetic analysis was performed using both traditional compartmental modeling and a population pharmacokinetic approach to analyze the tissue and plasma drug concentration data.
Results: BUP increased the risperidone plasma (5.9-fold, $P<0.01$) and brain (2.2-fold, $P<0.01$) area under the drug concentration vs. time curve (AUC), but did not alter the brain to plasma concentration ratio. SERT did not significantly change the plasma AUC of RISP and 9-hydroxy-RISP, but increased the brain AUC of RISP and 9-hydroxy-RISP by 1.5-fold ($P<0.05$) and 5-fold ($P<0.01$), respectively. Interactions were also evaluated in the opposite direction. RISP did not produce significant alterations of plasma or brain concentrations of BUP. It increased the plasma AUC and elimination half-life (t1/2e) of desmethyl-SERT while having little effect on brain concentration of this metabolite.
Discussion: These results suggest that pharmacokinetic interactions exist among these three psychoactive drugs involving inhibition of drug metabolizing enzymes in the liver and/or P-gp and other drug transporters present in the BBB. The mechanisms and consequences of these interactions require further study and confirmation in humans to establish any clinical relevance. This study was conducted in accordance with the NIH laboratory animal use guidelines.

119. A Data-Driven Approach for Understanding the Stages of Schizophrenia

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Sponsor: Sam Siris

Background: Although criteria exist for the diagnosis of schizophrenia, there is a need to more effectively communicate disease course to patients and families. In other areas of medicine, such communication is facilitated by well-established definitions of acute, stable, remitted, and recovered states. Defining or characterizing the stages of

schizophrenia may advance care by (1) assessing progress of treatment; (2) identifying barriers to improvements; (3) enhancing communication and setting expectations for patients, families, and caregivers; and (4) identification of stage-specific aspects of treatment efficacy for certain interventions. We explored the hypothesis that a definable and characteristic sequence of changes in symptoms exists from the acute to the remitted state in schizophrenia.
Methods: Three databases were used to characterize patients with schizophrenia in the acute, stable, and remitted states. Study 1 was a double-blind, international, 6-week study of subjects with a recent acute exacerbation. Studies 2 and 3 were both international, one-year studies in symptomatically stable adults with chronic disease. Remitted patients were identified from studies 2 and 3 by applying recently defined research criteria for remission (Andreasen et al. Am J Psychiatry. 2005;162:441-449). The three patient populations (Acute, Stable, and Remitted) were compared by the 30 Positive and Negative Syndrome Scale (PANSS) item analysis and factor scores.
Results: These three patient groups (Acute, Stable, and Remitted) were characterized by distinct symptom profiles. Observation of PANSS item data led to the identification of 4 groups of symptoms referred to here as: Grossly Disorganized Behavior (ie, excitement/hostility), Severely Disturbed Reality Testing (ie, positive symptoms), Interpersonal Relatedness, and Retardation Depression. Both groups of stable patients (Study 2 and Study 3 at baseline) had similar profiles, with very low mean scores (absent to mild) for all symptoms; Interpersonal Relatedness items rated the highest (mild). Both groups of Remitted patients (identified from Study 2 and Study 3 after treatment) were also similar. In the Remitted patients, Interpersonal Relatedness symptoms remained the most prominent, but were lower in severity than those of the Stable populations. The Acute population differed from Stable patients in pattern and severity, with markedly higher ratings for symptoms of Grossly Disorganized Behavior and Severely Disturbed Reality Testing. Interpersonal Relatedness symptoms, however, had a level of severity similar to that of Stable patients, suggesting these symptoms do not change in patients transitioning from the acute to the stable state.
Discussion: This data analysis provided useful information to begin characterizing the stages of schizophrenia. Future efforts will focus on ways to include measures of cognition, functioning, stress tolerance, and physical health as well as the identification of crucial issues relevant to various stage transitions. This patient characterization is part of a larger initiative to bring together representatives from treatment teams, caregivers, patients, and advocacy groups to (1) refine data-based definitions of characteristic features of the stages of schizophrenia; (2) develop a simple tool for patient assessment; and (3) create a tool to help clinicians communicate more effectively with patients, families, and caregivers. Source of Funding: Janssen, LP.

120. Deficits in PFC Activity in Schizophrenia Patients During the Maintenance and Response Selection Phases of Working Memory

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Sponsor: John Krystal

Background: Working memory (WM) can be divided into encoding, maintenance and response phases. Preclinical studies suggest that dopamine D1 and NMDA glutamate receptors are essential for the maintenance phase of WM, while dopamine D2 receptors contribute to the response selection phase. This study used event-related functional magnetic resonance imaging (fMRI) to measure prefrontal activity in medicated schizophrenia patients and healthy comparison subjects during WM encoding, maintenance and response phases.
Methods: Schizophrenic patients (n = 14) and matched healthy subjects (n = 12) completed fMRI testing. WM performance was studied at two levels of difficulty (2-item vs. 4-item). An initial encoding peak and a trough preceding a response peak were identified for each subject in predefined anatomical regions of interests within the prefrontal cortex. Peak percent change from baseline during each period of interest was the dependent variable.
Results: Prefrontal activity during the encoding period did not differ between schizophrenia patients and healthy subjects. However, patients exhibited reduced prefrontal activity relative to the healthy subjects in the maintenance and response phases of WM. Within a region, activation on the first peak and the trough significantly influenced response activation, but these effects did not fully account for the response selection phase deficits in schizophrenic patients. Diagnosis and medication influenced response activation in a complex manner.
Discussion: These data suggest that schizophrenia patients show deficits in prefrontal cortex during the maintenance and response selection phases of WM, while prefrontal activity during encoding is intact. The maintenance phase deficits are consistent with hypothesized functional deficits in D1 and NMDA receptors in prefrontal cortex. The response selection phase deficits may reflect, in large part, a detrimental effect of neuroleptic medication.

121. Reduced Hospitalisation in Patients with Schizophrenia Initiated with Risperidone Long-Acting Injection (LAI): 3 Month Follow-Up from the e-STAR Database

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Sponsor: Lennart Wetterberg

Background: e-STAR (electronic-Schizophrenia Treatment Adherence Registry) is an ongoing, long-term, international, observational study of patients with schizophrenia who commence treatment with a new anti-psychotic. The objectives of this present analysis are to compare hospitalisation rates, use of concomitant medications and functioning in patients with schizophrenia, before and after commencing treatment with risperidone LAI.
Methods: Data are entered into the web-based tool via a secure privacy protected system during regular routine clinical care. There are no inclusion or exclusion criteria. Patient demographics and resource use are recorded for each patient for 12 months retrospectively, and 2 years prospectively. Patient outcomes include Clinical Global Impression - Severity (CGI-S) and Global Assessment of Functioning (GAF). Adverse events data are also collected. Data collection and patient follow-up continue whether or not patients remain on their initial treatment.
Results: Data reported here are for the first 3 months in which 1,728 patients started treatment with risperidone LAI (given as 2-weekly intramuscular injections) in Germany (n=1370) and Australia (n=358). Mean age was 41.8 (s.d. 13.5) years, 58.6% were male, and mean duration of illness at switch was 10.4 (s.d 9.5) years. The majority of patients had a diagnosis of schizophrenia (78.4%) whilst 18.1% had schizoaffective disorder. The starting doses of risperidone LAI were 25 mg (65% patients), 37.5 mg (22%) or 50 mg (13%) and 80% of all patients remained on their original dose. Compared to the 3 months prior to risperidone LAI the proportion of patients hospitalised decreased significantly from 36.5% to 24.3% ($p<0.001$). The average number of days in hospital significantly decreased from 13.3(s.d. 23.5) to 8.5(s.d. 21.2) with a mean change of -4.9 days per patient ($p<0.001$). There was also a reduction in the mean number of hospital stays per patient from 0.45 (s.d 0.69) to 0.30 (s.d. 0.59) ($P<0.001$). The proportion of patients hospitalised for more than 30 days also fell from 18.1% to 10.1% ($p<0.001$). The proportion of patients requiring other concomitant non-antipsychotic medication decreased significantly after treatment with risperdone LAI.(54% to 46%; $p<0.001$). The use of anticholinergics decreased from 15.1% to 11.2% ($p<0.001$) and benzopdiazepines decreased from 21.2% to 17.1% ($p<0.001$). At baseline 22% of patients were considered se-

verely or extremely severely ill (by CGI-S) compared with 8% at 3 months post the new treatment. Functioning (by GAF) also improved significantly from 45.8 (s.d. 15.4) at baseline to 53.9 (s.d. 16.3) 3 months later ($p<0.001$).
Discussion: These first follow-up data from e-STAR suggest that schizophrenia patients treated with risperidone LAI, as part of their routine clinical management, experience a reduction in hospitalisations, concomitant medications and improvement in functioning. More data are being accrued to explore this further.

122. Natural Prepulse Inhibition Deficits in Brattleboro Rats Respond to Valproic Acid Augmention of Clozapine: Further Support for the Predictive Validity of this Animal Model
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Background: The Brattleboro rat (BRAT) is a Long Evans derived rat with a single gene mutation resulting in deficient vasopressin production. BRATs are known to have cognitive abnormalities similar to those seen in schizophrenia patients. Our laboratory discovered that BRATs exhibit natural deficits in prepulse inhibition (PPI) of their startle reflex which is similar to those exhibited by schizophrenia patients. We subsequently demonstrated that PPI deficits in BRATs can be reversed with antipsychotics in a manner that differentiates typical from atypical agents and that also models their therapeutic time course in which effects are stronger after chronic administration. This suggests that the BRAT is a useful genetic model of sensorimotor gating deficits associated with schizophrenia as well as a useful predictive screen for putative antipsychotics. A truly useful model of antipsychotic efficacy would not only have predictive validity for antipsychotic monotherapy but also for more complex strategies such as antipsychotic drug augmentation with non-antipsychotic drugs. It has been demonstrated in several studies that valproic acid does not have antipsychotic efficacy by itself but that it can augment the efficacy of atypical antipsychotics (for review see Citrome, Psychopharmacol Bull. 2003;37 Suppl 2:74). In order to further explore the predictive validity of BRATs, we studied the effects of valproic acid on BRAT PPI deficits by itself and in combination with clozapine.
Methods: We administered several doses of valproic acid (50-200 mg/day) subcutaneously to BRATs one time or repeatedly for many days. In addition, we administered clozapine (5 mg/kg) by itself or in combination with valproic acid. These animal studies have been 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.
Results: Valproic acid (50, 200 mg) administered by itself acutely or chronically did not significantly modify PPI deficits in BRATs. In contrast, valproic acid in combination with clozapine produced a significantly greater increase in BRAT PPI compared to clozapine alone.
Discussion: These results suggest that in addition to modeling the efficacy of monotherapy antipsychotic treatments the BRAT also models the efficacy of established antipsychotic augmentation strategies. This strengthens the apparent predictive power of these rats as a screening tool for potential new antipsychotic treatments and strategies.

123. Corollary Discharge Failure in Schizophrenia is Related to Severity of Auditory Hallucinations: fMRI Evidence
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Background: Communication between frontal lobes, where speech and verbal thoughts are generated, and temporal lobes, where they are perceived, may be dysfunctional in schizophrenia. The efference copy/corollary discharge mechanism may act to suppress cortical responses to thoughts and self-produced speech. Dysfunction of this mechanism may result in the misperception of inner speech and inner experiences as coming from an external source. This would result in "voices", or auditory hallucinations. We used functional magnetic resonance imaging (fMRI) data to localize more precisely the brain regions suppressed during talking in healthy controls, the lack of suppression in these regions in schizophrenia and its association with auditory hallucinations.
Methods: DSM-IV schizophrenia patients (n=21) recruited from the community and the VA hospital, and sex- and age-matched healthy control subjects (n=21) recruited from the community. FMRI data were collected while participants uttered the syllable [a] aloud (Talk) and then listened to it played back through headphones (Listen). Both talking and listening were timed to occur only during the silent periods of clustered acquisition sequences.
Results: Consistent with the successful action of a corollary discharge mechanism suppressing auditory responsiveness during talking, activity in middle temporal gyrus (MTG) was greater during Listening than Talking in healthy controls. In patients, there was no suppression of MTG activation during talking. Instead, patients had more auditory cortical activation during talking than listening. Patients with the greatest MTG activation during talking compared to listening had the most severe auditory hallucinations. Lack of suppression of auditory activity in brain stem structures (inferior colliculus and thalamus) during talking was also related to the severity of auditory hallucinations.
Discussion: These data suggest that the corollary discharge mechanism that works to suppress auditory responses during talking, and perhaps during thinking and internal language-related experiences, is dysfunctional in patients with schizophrenia. This dysfunction may contribute to auditory hallucinations.

124. Mice Deficient for an Immediate Early Gene Show a Heightened Response to Environmental Stressors Consistent with a Schizophrenia Phenotype
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Sponsor: C. Robert Cloninger

Background: Psychotic illnesses such as schizophrenia are characterized by disturbances in the perception of, and response to, events in the environment. Immediate early gene transcription factors (IEG-TFs) are activated in the brain in response to environmental stimuli including social interactions, emotional and stressful situations, and psychotherapeutic interventions such as antipsychotic medications and electroconvulsive seizure. As transcriptional regulators IEG-TFs are poised to mediate the molecular response of the brain to changes in the environment. IEGs are thus unique candidates for influencing both the genetic and environmental contributions to psychiatric illnesses such as schizophrenia. A member of this family is a particularly compelling candidate gene as it is regulated by two independent proteins that have been implicated in the pathogenesis of schizophrenia, and itself maps to a major locus for schizophrenia.
Methods: Mice deficient for an IEG (IEG-/- mice) were generated using a targeted deletion strategy and back-crossed to C57BL/6 mice for greater than 10 generations. IEG-/- and wildtype littermate controls were evaluated in the following behavioral tests: 1 hour open field activity, acoustic startle response, reactivity to handling, cagemate social interaction, and the resident intruder test of aggressive behavior. Blood was obtained via retro-orbital bleed at circadian nadir for baseline corticosterone levels and, 1 week later, following handling. Corticosterone levels were determined by radio-immuno assay. Statistical analyses were performed using the Systat and SPSS programs.
Results: We report that IEG -/- mice behave abnormally in response to a range of environmental stressors that are consistent with a schizophrenia phenotype. Like other mouse models of psychosis, IEG -/-

mice are hyperactive in a novel environment, and they display a heightened startle response to an auditory stimulus that fails to habituate to repeated exposure. We demonstrate that IEG -/- mice are more reactive to the mild stress of handling, and their behavioral response is paralleled by augmented release of corticosterone (the murine equivalent to cortisol) compared with wildtype littermates. Also, like patients with schizophrenia, IEG-/- mice show disruptions in social behavior, including abnormalities in social investigatory behavior and increased levels of aggression.
Discussion: We have found that mice lacking an IEG show abnormalities in the behavioral and physiologic response to environmental stimuli which are consistent with a schizophrenia phenotype. Hyperactivity and an accentuated startle response that fail to habituate are hallmarks of a schizophrenia phenotype in mice. Furthermore, the accentuated stress response seen in IEG -/- mice is consistent with the increased sensitivity to psychosocial stress and abnormalities in cortisol regulation seen in patients with schizophrenia. In addition, IEG-/- mice show abnormalities in social interactions, including a heightened aggressive response toward a foreign intruder mouse, a phenotype analogous to the increased rates of aggression seen in inadequately-treated schizophrenia patients. These results indicate that a protein induced by environmental stress is, in fact, required for the behavioral and physiologic response to stress. Furthermore, these findings suggest that defects in the function of this IEG in humans may contribute to neuropsychiatric illnesses such as schizophrenia.

125. A Pilot Study Assessing the Effects of Dar-0100 (A Dopamine D1 Full Agonist) on Regional Brain Activity and Task-Specific Activation in Patients with Schizophrenia

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Background: Studies in non-human primates have shown that dopamine D1 receptors play an important role in memory and cognition. There are several studies providing evidence that supports the hypothesis that dopamine D1 agonists can reverse performance deficits in either aged non-human primates, or in primates with lesions to dopamine systems. Unfortunately, no centrally-active selective D1 agonists have been available to test such ideas. This study utilizes the first full D1 agonist, dihydrexidine (DAR-0100). DAR-0100 has somewhat higher D1 affinity, and greater D1 selectivity, than ABT-431, the only other D1 agonist to be given to humans. Availability of this drug has permitted us to conduct an initial pilot study assessing the safety and efficacy of low doses of DAR-0100 on prefrontal cortical blood flow and cognitive function in patients with schizophrenia.
Methods: The effects of DAR-0100 on resting blood flow in the prefrontal cortex and neural activity in regions involved in working memory are being evaluated. A within subject cross-over design is being used in 20 adults (18-65 yrs of age) with SCID diagnosed schizophrenia. Subjects are outpatients whose stable doses of selected antipsychotic medications still leave a moderate level of remaining negative symptoms. Following a screening visit, subjects are admitted for a 48 hour inpatient hospitalization. Each morning at 8 am they are scanned on a 3T MRI scanner for resting perfusion, followed by a BOLD fMRI scan during the n-back working memory task. They then receive 20 mg of DAR-0100 (or placebo) sc over 15 minutes. Over the next 45 minutes they have intermittent MRI scans of perfusion and BOLD activity during the working memory task. Response data and serum levels are collected. A repeat MRI scan is done at 6 pm, without any infusions. The following morning they have a repeat of the Day 1 schedule, and receive either DAR-0100 or placebo (whichever was not given on Day 1). Within day, as well as between day, comparisons will be made to test for potentially increased rCBF with DAR-0100.
Results: We have studied 15 of an anticipated 20 subjects (enrollment completion date Sept 1). Data are still blind. The doses of placebo and DAR-0100 have been well-tolerated, with no SAE's. Two subjects had mild dizziness and nausea one hour after dose. Full unblinded side effect, cognitive performance, and brain imaging data will be presented at the meeting.
Discussion: This first proof-of-concept study may have important implications for the treatment of cognitive and memory deficits in schizophrenia and other CNS disorders.

126. Neuropsychological Deficits in Non-Smokers with Schizophrenia: Effects of a Nicotinic Antagonist

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Background: Biochemical, physiological and genetic evidence suggests dysregulation of central nicotinic acetylcholine receptor (nAChR) systems in schizophrenia, which may contribute to neuropsychological dysfunction and the high rates of smoking in this disorder. To evaluate the effects of nAChR blockade on neuropsychological performance in schizophrenia without the confounding effects of cigarette smoking, we compared neuropsychological performance in schizophrenia and healthy control non-smokers after pre-treatment with the nAChR antagonist mecamylamine (MEC).
Methods: Using a within-subjects, counterbalanced design, schizophrenia (n=13) and control (n=15) non-smokers (both former and never smokers) were pre-treated for three days with MEC (0.0, 5.0, and 10.0 mg/day). Subjects performed repeated neuropsychological assessments including visuospatial working memory (VSWM), Continuous Performance Test (CPT), Wisconsin Card Sorting Test (WCST), Word Serial Position Test (WSPT) and Stroop Color Word Test (SCWT) during three sequential test sessions per week over three test weeks.
Results: We found significant main effects of schizophrenia on: VSWM 30 and 60 second delays (p's<0.01), CPT (% Hit Rate, Reaction Time, Variability Index; p<0.01 for all outcomes), WCST (p<0.01 for all outcomes) and Word Serial Position Test (p<0.01). There were no main effects of repeated test administration (Session) or MEC dose on any of these outcomes, and no significant 3-way (Diagnosis x Session x MEC Dose) interactions. In exploratory analyses, subjects with schizophrenia who were never smokers (n=6) performed worse than former smokers (n=7) on the majority of tasks.
Discussion: Our results suggest that the nAChR antagonist MEC does not significantly alter endogenous neuropsychological deficits in nonsmokers with schizophrenia. Furthermore, differences in baseline performance between former and never smokers with schizophrenia suggest these findings are trait effects related to distinct schizophrenia subtypes, and independent of smoking. The significance of these findings in relation to differential expression of nAChRs in schizophrenic non-smokers versus smokers is discussed.

127. Exploring the Evidence for a Relationship Between Symptomatic Remission and Improvement in Functioning in Patients With Schizophrenia

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Sponsor: George Simpson

Background: Schizophrenia is a chronic, relapsing illness necessitating life-long treatment. Once a patient's illness is stable, maintenance therapy is required in order to decrease the risks of relapse and rehospitalization and to increase the potential for remission. A recently published proposal defining remission in schizophrenia illustrates

increasing interest in measures of wellness as targets of treatment outcome (Andreasen et al. Am J Psychiatry. 2005;162:441-449). Long-acting risperidone, the first long-acting, injectable atypical antipsychotic, has recently been studied in a long-term maintenance trial. Study results demonstrated low rates of relapse and rehospitalization. This post-hoc analysis of the database examined how meeting remission criteria corresponded to various ratings of patient status in order to address the important question of whether a symptom-based remitted state corresponds to overall improved patient functioning.

Methods: Data are from a prospective, randomized, double-blind, multicenter, international, 52-week trial involving 323 patients with schizophrenia or schizoaffective disorder as per Diagnostic and Statistical Manual of Mental Disorders, 4th ed., criteria. Stable patients received long-acting, injectable risperidone (25 or 50 mg every 2 weeks). Remission criteria were defined as absent to mild symptoms on all 8 core Positive and Negative Syndrome Scale (PANSS) items for ≥6 months. Patients were evaluated using the PANSS and Clinical Global Impressions of Severity (CGI-S) scale on weeks 0, 4, 8, 12, 18, 24, 30, 36, 42, and 52/endpoint. Patients' functioning was assessed using the Personal and Social Performance (PSP) and Strauss-Carpenter Level of Functioning (LOF) scales at weeks 0, 24, and 52/endpoint. Safety was assessed at each visit via reporting of treatment-emergent adverse events (AEs).

Results: Scores are reported as means (± standard deviation [SD]). The mean PANSS total score at baseline was 66.5 (16.4). Although patients were considered stable at entry, 61.4% (n = 194) were not remitted (severity component only for all 8 items) at baseline. Among these patients, 21.6% (n = 42) met remission criteria (severity and duration) during the study; 90.0% of these remitted patients completed the study. Remitted patients experienced a low rate of protocol-defined relapse (n = 1; 2.4%); significant improvement in mean PANSS total score (73.3 ± 10.4 to 53.1 ± 10.7; $P < 0.001$); CGI-S improvements at endpoint (not ill to mildly ill; 24.4% to 88.1%); and significant improvements in mean PSP scores (60.6 ± 14.1 to 71.5 ± 10.9; $P < 0.001$) and LOF (22.0 ± 5.2 to 23.5 ± 5.0; $P = 0.05$) scores. Some improvements were noted in nonremitted patients, but to a much lesser extent. The most commonly reported AEs (≥15%) were: headache (26%) and insomnia (26%) among remitted patients; and psychiatric disorder not otherwise specified (28%), insomnia (26%), anxiety (17%), and headache (16%) among nonremitted patients.

Discussion: The primary results of this 1-year maintenance study of long-acting, injectable risperidone in stable patients with schizophrenia or schizoaffective disorder demonstrated low rates of relapse and rehospitalization. The findings reported here add to a growing body of data linking these remission criteria with improvement in functioning. Source of Funding: Janssen, LP.

128. Appraising Neurocognitive Endophenotypes for Schizophrenia

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Sponsor: Pedro L. Delgado

Background: Although genetic influences on schizophrenia are well established, localization of genes responsible for this illness remains elusive. Given evidence that genes predisposing to schizophrenia may be transmitted without expression of the clinical phenotype, strategies focused on developing endophenotypes, markers of processes mediating between genotype and phenotype, have gained popularity. While several neuropsychological measures are proposed to be endophenotypes for schizophrenia, few studies have systematically assessed batteries of neurocognitive tests to determine which tests are most sensitive to liability for the illness.

Methods: Here, we performed 214 comprehensive neuropsychological assessments on individuals with schizophrenia (n=55), their first (n=40) and second (n=29) degree relatives and non-related subjects (n=41). All participants were Latino individuals from 46 extended pedigrees living in Costa Rica, Mexico, or the United States. Families were recruited if they contained a sibling pair with schizophrenia and at least two grandparents of Mexican or Central American origin. Those neurocognitive measures found to be significantly heritable ($p<0.01$) were entered into hierarchical analyses schedule to determine which tests covary with the degree of genetic relationship with affected individuals.

Results: Three neuropsychological measures were found to meet each of the criteria for an endophenotype: processing speed, spatial working memory, and verbal episodic memory. To assess the specificity of these endophenotypes for schizophrenia, performance on these measures by probands were compared to family members with bipolar (n=21) and unipolar (n=16) affective disorders. The bipolar group was as impaired on the measures of processing speed and spatial working memory as the schizophrenia group and less so for the verbal episodic memory measure. In contrast, the unipolar group was impaired only on the speed of processing measure relative to healthy subjects.

Discussion: As these measures contributed uniquely to discriminate individuals at varying risk for schizophrenia, our findings imply multiple independently inherited elements to the liability for the illness. Furthermore, we suggest that some of these measures may be sensitive to biologic processes disrupted in both schizophrenia and bipolar disorder.

129. Placebo-Controlled, Add-On Trial of CX516 (Ampakine) in Schizophrenia

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Background: AMPA receptor positive modulators (ampakines) have demonstrated efficacy in several animal models of learning and memory. CX516, a relatively low-potency ampakine, has been shown to increase the peak and duration of glutamate-induced AMPA receptor-gated inward currents and to increase hippocampal activity *in vivo* during behavioral training in rats. In one preliminary placebo-controlled trial in 19 clozapine-treated schizophrenia patients, CX516 improved measures of attention and memory, which persisted at 2-week follow-up.

Methods: 105 schizophrenia patients treated with a stable dose of clozapine (n= 53), olanzapine (n=39) or risperidone (n=13) were randomly assigned to CX516 900 mg tid or placebo for four weeks. A cognitive battery, including WCST, CVLT, Letter and Category Fluency , Degraded Stimulus CPT, Faces subtest from WMS-III, Letter-Number Sequencing, Trail Making A and B and Grooved Pegboard was administered at baseline, week 4 and at follow-up at week 8. Clinical ratings including PANSS, SANS, Calgary Depression Rating Scale, GAS and Quality of Life Scale were administered at baseline and weeks 2, 4 & 8. Safety assessments including vital signs and the SAFTEE were completed weekly; routine laboratory and EKG were performed at baseline and week 4.

Results: 121 patients were screened, 105 were randomized and 92 completed the four-week trial. Week 8 follow-up data were available on 87 patients. Effects of CX516 on cognitive performance and clinical ratings during the four-week trial and at follow-up will be presented in addition to data on safety and tolerability.

Discussion: These results represent the first large trial of add-on treatment with a positive AMPA modulator for cognitive deficits in schizophrenia.

130. The Trajectory is The Story: Cortical Gray Matter Changes During Adolescence In Childhood Onset Schizophrenia Evolve Into The Adult Pattern By Age 25

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Sponsor: Judith Rapoport

Background: Childhood onset schizophrenia (COS) shows a robust and progressive gray matter (GM) loss during adolescence, which was shown to proceed in a parietal-frontal (back to front) direction. We wanted to see whether the gray matter (GM) loss pattern was typical of our larger group of subjects, and if it eventually mimicked the pattern seen in adult onset schizophrenia (AOS).
Methods: Anatomic brain MRI scans were obtained from 70 COS cases rescanned every two years, thus covering ages 7 through 26 (160 scans). These were compared with MRI scans from 72 matched healthy controls (165 scans). Using a novel, fully automated cortical thickness analyses method, mixed model statistical analyses, and controlling for the mean cortical thickness, GM changes were mapped across the entire cortex across time.
Results: From childhood through adolescence, COS subjects showed profound GM loss beginning in the superior parietal and frontal regions which progressed to prefrontal and temporal cortices (back to front pattern). As the subjects grew older (after age 20), the GM loss remained primarily in the prefrontal and superior temporal cortices
Discussion: These findings, which extend the robust and back to front nature of cortical GM loss during adolescence to a larger group of COS, also suggest that with advancing age, the GM changes in COS gradually evolve to the pattern seen for the AOS, further establishing the continuity between the childhood and later onset schizophrenia.

131. Reelin (RELN) Promoter Hypermethylation In Gaba Neurons In Schizophrenia

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Sponsor: Erminio Costa

Background: Evidence is accumulating that in schizophrenia, cognitive defects associated with pyramidal neuron firing appear to relate to a GABAergic neuronal pathology. The Reln and GAD67 mRNA down-regulation in GABAergic neurons of post mortem brains of patients with schizophrenia is firmly established. We have shown that methionine injection into mice decreases reelin and GAD67 expression through a mechanism that likely involves hypermethylation of the corresponding promoters. Among DNA methyltransferases (Dnmt), Dnmt1 is highly expressed in post-mitotic neurons and is increased in those same GABAergic neurons in which the Reln and GAD67 mRNAs are decreased in PFC of patients with schizophrenia. This inverse relationship supports the hypothesis that Dnmt1 may function to establishing the DNA methylation patterns in GABAergic neurons. Moreover, following protracted methionine treatment, there is increased binding of the repressor protein MeCP2 to the reelin and GAD67 promoters consistent with this hypermethylation.
Methods: To examine this hypothesis in more detail, we have mapped the methylation pattern of the human Reln promoter in DNA isolated from post-mortem brain tissue from schizophrenia and control patients from two different brain collections.
Results: Analysis of the data shows that site selective increases in cytosine methylation exist in the Reln promoter in DNA isolated from schizophrenia patients that are not methylated in non-psychiatric subjects. That is, certain positions were more consistently methylated in schizophrenia patients as compared to non-psychiatric subjects. For example, results showed that the number of methylated bases upstream of the RNA start site in SZP is significantly greater than the number present in clones from NPS group. Further analysis found that there was a significant difference ($p<0.001$) in the frequency of the number of methylated bases at sites -133 and -138 (relative to the RNA start site) between SZP and NPS. For example, within the Stanley Foundation set, there were 0.4 ± 0.3 methylated bases at site -138 for the NPS group, while with the SZP there were 3.4 ± 0.5 bases per total sample set. At -133, the NPS showed 0.3 ± 0.2 while the SZP exhibited 3.2 ± 0.5. These differences were significant in both cohorts at the level of $p < 0.001$. Gel shift analyses demonstrate that extracts prepared from neuronal progenitor cells that do not express reelin, show increased repressor binding to the methylated sites.
Discussion: Collectively, these results are consistent with the concept that the down-regulation of mRNAs in GABAergic neurons of schizophrenia brain may be the result of Dnmt1-mediated promoter hypermethylation and heterochromatin formation. The findings suggest that promoter methylation plays an important role in the reduced expression of reelin in schizophrenia, and that methylation might promote the binding of repressor factors to functionally defined cis-acting elements hence compromising promoter function. It also offers a mechanism by which multiple genes may be affected by a single mechanism which can also explain discrepancies between disease status and the epigenome of monozygotic twins. These studies should provide the foundations for a new pharmacology for the treatment of schizophrenia and a better appreciation of the role of GABA in the pathogenesis of schizophrenia symptomatology.

132. The Consortium on the Genetics of Schizophrenia (COGS): Preliminary Heritability Analyses of Endophenotypic Measures for Schizophrenia

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Sponsor: Michael Green

Background: The discovery of genes predisposing to complex diseases, such as schizophrenia, has proven to be very difficult. The genetic exploration of endophenotypes, or discrete, genetically-determined phenotypes that may be part of a complex illness, may be a powerful strategy for understanding the genetics of schizophrenia. As such, we have undertaken a study to characterize the genetic architecture (i.e., heritability, genetic correlations, etc.) of several endophenotypic measures in an effort to determine which of these measures are (a) likely to reflect the same underlying neurobiological processes and (b) indicate genetic transmission of risk for schizophrenia. Endophenotypic variation can be used to characterize subtypes of schizophrenia. We have focused our efforts on automatic processing measures of P50 suppression, prepulse inhibition (PPI) of the startle response. We also examined effortful, controlled processing measures of the antisaccade task, the Continuous Performance Test (CPT), the California Verbal Learning Task (CVLT, a measure of verbal memory), and the Letter-Number Span (LNS, a measure of working memory). The following domains of the Pennsylvania Neurophysiological Battery have also been employed: Abstraction and Mental Flexibility, Face Memory, Spatial Memory, Spatial Processing, Sensorimotor Dexterity, and Emotion Recognition.
Methods: We have currently collected 106 schizophrenic probands and their immediate family members for a total of 411 individuals that have been assessed for the previously mentioned endophenotypes. The genetic analysis software package SOLAR was used to assess the genetic heritability of each of the endophenotypic measures, as well as the environmental and genetic correlations between the endophenotypes. A correction for ascertainment bias was employed.
Results: We analyzed heritability of the controlled, volitional measures. The Antisaccade Task, the LNS, and the degraded stimulus version of the CPT were the most heritable of the endophenotypes stud-

ied with heritabilities of 0.55, 0.46, and 0.40, respectively ($p<0.0001$). The primary variables from the identical pairs version of the CPT were found to be significantly heritable with heritabilities of 0.29 and 0.34 ($p<0.01$). The primary measure of the CVLT was found to be moderately significant with a heritability of 0.17 ($p<0.05$). Several measures from the Penn Battery, Spatial Processing, Sensorimotor Dexterity, and Emotion Recognition, were also found to be significantly heritable with heritabilities in the range of 0.29-0.49. Age was found to be a highly significant covariate in nearly all analyses, and gender and years of education were found to be significant covariates in many analyses. Significant environmental and genetic correlations were also observed between many of the endophenotypic measures.
Discussion: Although these analyses are preliminary, we have found that variation in these endophenotypes is quite heritable as predicted. Genetic correlations between several of the endophenotypes provide some evidence for pleiotropic effects. Endophenotypes from the COGS project appear to be important measures in determining the genetic basis of schizophrenia.

133. Convergence of Cognitive Improvement with Development of Clinical Remission in Schizophrenia
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Background: A consensus definition of remission in schizophrenia has been recently proposed which systematically describes an essentially symptom-free clinical state. However, it is also established that cognitive impairments are associated with functional deficits in schizophrenia and, therefore, that cognitive enhancement may be required in order to improve functional outcomes. We examined the development and maintenance of sustained symptomatic remission and the association between substantial neuropsychological improvements and development of remission in a large sample of patients with schizophrenia whose medication was switched to ziprasidone.
Methods: One hundred and seventy-seven patients were switched from their previous treatment with risperidone, olanzapine, or conventional anti psychotics to open-label ziprasidone treatment. One hundred and thirty seven patients were not in remission and 40 were in remission at study baseline (using cross-sectional remission criteria). We rated their symptoms with the PANSS at baseline prior to the switch and after 6 weeks and 6 months of treatment. We also performed a comprehensive neuropsychological assessment, at the same time-points, which was used to generate a composite score which was then correlated with symptomatic remission and examined for improvements in the same time frame.
Results: Of the 40 patients meeting cross-sectional remission criteria at baseline, 34 (85.7%) sustained their remission for 6 months. Of the 137 patients not in remission at baseline, 55 (40%) met the symptomatic severity criteria for remission by 6 weeks. At 6 months 50 of 137 (36.5%) patients met full remission criteria. Therefore a total of 84 of the original 177 subjects (47%) were in sustained remission at 6 month endpoint. Fifty nine patients (34%), improved by at least 1.0 SD in their cognitive performance. There were no baseline differences in cognitive performance between those patients who were and were not in remission. Attainment of clinical remission was not correlated with baseline cognitive performance or cognitive improvements in this study. However, 21 of the 55 (38%) patients who achieved clinical remission also improved by at least 1.0 SD in their cognitive performance
Discussion: After a switch from previous treatment to open-label ziprasidone close to half of patients with schizophrenia experienced sustained clinical remission over 6 months and nearly 40% of patients achieving remission experienced a substantial concurrent cognitive improvement. Thus there were a substantial proportion of patients who manifested clinical remission and clear cognitive improvements. Since cognitive performance at baseline and cognitive changes did not converge overall with development of clinical remission, later research will be required to determine which aspects of improvement (clinical remission and/or cognitive improvements) are predictive of functional improvements.

134. Cognitive and Functional Improvement with Long Term Treatment with Long-acting Risperidone
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Background: Cognitive deficits in schizophrenia are common, wide ranging, and known to have considerable functional relevance. While patients receiving atypical antipsychotics, including risperidone, have shown evidence of improved cognition, it is not clear if these improvements are sufficient to be functionally relevant. One of the major issues regarding the possible functional relevance of the previous results is the typically short duration of many of these trials examining treatment effects. This report examines cognitive improvements and subsequent effects on real-world functioning in a 12-month study of long-acting risperidone.
Methods: Data were derived from a prospective, randomized, double-blind, international, 52-week study. Clinically stable patients ($N = 282$ with baseline and post baseline assessments) with schizophrenia were randomized from stable doses of prior oral antipsychotic to bi-weekly injections of long-acting risperidone at a dose of 25 or 50 mg. Patients received a computer-administered cognitive assessment test battery with alternate test forms at baseline, 3, 6, and 12 months. Domains assessed included processing speed, attention and impulsivity, working memory, verbal learning and memory, visual memory, executive function, and social cognition. Changes from baseline to endpoint were as analyzed for all of the cognitive assessments
Results: Significant improvements were noted in four of the seven cognitive domains. These included attention and impulsivity (total correct incongruent trials on the Flanker CPT ($p< 0.001$); word-list memory: total learning ($p< 0.001$); object working memory: mean difficulty level achieved ($p< 0.001$); and executive functioning (strategic target detection test: total efficiency; $p < 0.001$). The relationship between changes in these cognitive domains functional outcomes will be presented.
Discussion: Cognitive functioning improved significantly in multiple domains in previously stable patients switched to maintenance treatment with long-acting risperidone. Thus, even patients with minimal levels of psychotic symptoms appear to manifest cognitive improvement in long-term atypical antipsychotic treatment. Whether these changes have clinical relevance over a 12-month period will be explored, as this time period is longer than most previous trials with a substantial sample of patients with schizophrenia.

135. Cognitive Coping and Illusion of Control Can Both Modulate Hypothalamic-Pituitary-Adrenal Response to Pharmacological Activation
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Sponsor: Elizabeth Young

Background: The hypothalamic-pituitary adrenal (HPA) axis contributes to the deleterious impact of stress on emotional and physiological functioning. It helps mediate adaptation to environmental challenge and is sensitive to cognitive, emotional, and social aspects of organism-environment exchanges. Novelty, control, coping, and social buffering are significant modulators of HPA stress responses in animals and may be particularly salient to stress effects on health, but these factors have been difficult to study in humans. We have previously shown that a cognitive intervention (CI) designed to improve

cognitive coping, reduce novelty, and enhance sense of control could significantly reduce cortisol response to pentagastrin, a pharmacological HPA activator. We now sought to replicate this finding and determine whether the sense of control component of the intervention was necessary or sufficient to produce the modulation previously seen.
Methods: Healthy subjects were randomly assigned to one of four groups: (1) standard instructions, (2) full CI; (3) cognitive coping/novelty component of the CI; (4) illusion of control component alone. Instructions were administered prior to two admissions to a GCRC for our standard, two visit (placebo first) pentagastrin infusion paradigm.
Results: Preliminary analyses on 40 subjects (data collection continues) did not show a significant effect of the full CI on cortisol response to pentagastrin. However, both primary components (control and coping/novelty), administered separately, did significantly reduce the cortisol response ($p<.008$).
Discussion: These data document that brief psychological manipulations can significantly modulate activity in HPA activation paradigms. We produced similar reductions in cortisol using either (1) simple installation of a belief that one can control exposure to an activating agent, or (2) cognitive preparation that focused on drug side effects, reducing potential surprise and enhancing cognitive coping. It is currently unclear why the two components administered together appeared less effective. Further study of cognitive HPA modulators may lead to specific psychological techniques to combat the negative health effects of stress.

136. Mifepristone for the Prevention of Olanzapine-Induced Weight Gain in Rats

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Sponsor: Alan Schatzberg

Background: Multiple animal models have been designed to help understand the physiological factors of obesity, including genetic models (fa/fa rats), induction of obesity through high fat diets, and through administration of atypical antipsychotics. Adrenal glucocorticoids appear to be important in the etiology of many types of obesity including dietary, hypothalamic and genetic forms of obesity (Bray et al., 1990). Adrenalectomy prevents hyperphagia and reduces fat synthesis in animal models of obesity (Marchington et al., 1986). Preliminary work indicates that blocking the type II glucocorticoid receptor with mifepristone reverses genetic obesity in young fa/fa Zucker rats (Langley, S.C. and D.A. York, 1990). After 15 days of treatment with mifepristone, 5-week old obese Zucker rats resembled lean vehicle rats in terms of body composition. In addition, dietary obesity in Osborn-Mendel rats also appears to be reversed by mifepristone (Okada, S., et al, 1992). Mifepristone (RU 486) is both a potent type II glucocorticoid antagonist and a progesterone antagonist. Given the evidence from prior studies that mifepristone may reverse genetic and dietary obesity in rodent models, there may be a role for mifepristone in preventing or reversing antipsychotic induced weight gain as well. Using a model of olanzapine-induced weight gain in rats, the objectives of these experiments were to (a) test whether mifepristone reverses olanzapine-induced weight gain, and (b) test whether mifepristone prevents olanzapine-induced weight gain.
Methods: Experiment 1: Adult female Charles-River rats received olanzapine, 1.2mg/kg, BID or vehicle (control) for 34 days; then received olanzapine, 1.2mg/kg, BID, plus mifepristone, 10mg/kg, 30mg/kg, or 100mg/kg, BID, or vehicle for 21 days. Experiment 2: Adult female Charles-River rats received olanzapine, 1.2mg/kg, BID, or olanzapine, 1.2mg/kg, BID, plus mifepristone, 10mg/kg, 30mg/kg, or 100mg/kg, BID, for 22 days. In both experiments, animals were dosed via gavage and had ad libitum access to a normal diet and water. Body weight was collected every 3 days and food consumption was measured daily. Abdominal fat was measured at termination.
Results: Experiment 1: Weight gain was significantly greater for the olanzapine group ($p<.01$) at day 35. The mifepristone + olanzapine groups lost a significant portion of the weight they had gained on olanzapine alone ($p<.0001$) from day 35-42, and average weights of the groups were not statistically different from controls at study end (day 55). Experiment 2: The olanzapine group gained significantly more weight compared to the mifepristone + olanzapine groups starting on day 3 ($p=.001$) and continuing through day 22 ($p=.0002$). Olanzapine treated rats had significantly more abdominal fat compared to rats in the mifepristone + olanzapine groups ($p<.0001$). Food consumption was significantly higher for the olanzapine group versus the mifepristone + olanzapine groups ($p=.0003$).
Discussion: Using an animal model of olanzapine-induced weight gain, these data demonstrate the utility of this model in testing a pharmacologic intervention, mifepristone, for the reduction and prevention of olanzapine-induced weight gain. We know from prior studies that corticosteroids are implicated in multiple forms of obesity, and that the use of mifepristone, a potent GRII antagonist, has been shown to reduce obesity associated with genetics and high fat diet in animal models. Further clinical studies must be conducted to test the efficacy and safety of mifepristone for the prevention of antipsychotic induced weight gain in humans.

137. Ovarian Steroids Suppress the Apoptosis-Inducing Protein JNK1 in the Dorsal Raphe Region of Macaques

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Sponsor: Daniel Dorsa

Background: We have shown that estrogen (E) and progesterone (P) regulate pivotal genes and proteins involved in serotonin neuronal function. In a recent study with Affymetrix microarrays, we found that E and P also regulate genes in the dorsal raphe area that may play a role in neuroprotection (Bethea and Reddy 2005 Psychopharmacology 180:125-140). JNK1 (c-jun n-terminal kinase 1) activates the caspase pathway and is considered a pro-apoptosis gene. JNK1 gene expression was markedly suppressed in the dorsal raphe region by ovarian steroid treatment of spayed monkeys in the microarray analysis and in a quantitative RT-PCR assay ($p < 0.007$). In this study, we questioned whether the change in JNK1 gene expression would be manifested at the protein level.
Methods: Female rhesus macaques were spayed for 3-6 months and then treated with placebo or E alone or E plus P via Silastic capsules s.c. or the selective estrogen receptor modulator (SERM) raloxifene in the diet as follows (n=4/group) (1) Spayed-empty Silastic capsule (2) E treatment for 1 mo (3) E plus P treatment for 1 mo (4) E treatment for 5 mos (5) E plus P treatment for 5 mos (6) Raloxifene treatment for 5 mos at 5-mg/kg-body weight daily Cytoplasmic extracts of the dorsal raphe region were processed for Western blotting as described previously (Smith et al., 2004 Neuropsychopharmacology 29:2035-2045). A cytoplasmic extract of the RN46 serotonin cell line served as the positive control The blots were probed with a mouse monoclonal antibody to human JNK1 p46 (Santa Cruz Biotech Inc). Densitometric analysis of signal bands was performed using NIH image gel plotting software and statistical analysis was conducted with ANOVA and Student-Newman-Keuls post-hoc pair-wise comparisons.
Results: One band at 46 Kd was observed in the RN46 extract. Four bands at ~39, 46, 56 and 80 Kd were observed in the dorsal raphe extract. There was a significance difference in the JNK1 p46 band between the treatment groups ($p<0.026$). JNK1 p46 was significantly suppressed with 1 or 5 mo of E treatment compared to placebo controls ($p<0.05$ SNK's). Addition of P did not alter JNK1 p46 compared to E only treatment. JNK1 p46 was slightly, but not significantly, suppressed by raloxifene treatment.
Discussion: JNK1 is believed to transduce a variety of extracellular stresses, UV radiation, heat shock, trophic factor deprivation, or cy-

tokines to selective cellular responses. In neurons, JNK1 plays a critical role in the pathogenesis of glutamate neurotoxicity. The ability of E, with or without P, to markedly decrease expression of JNK1 in the dorsal raphe region suggests that ovarian hormones may protect serotonin neurons from stress-induced death. Supported by MH62677, HD U54 18185 and RR00163

138. Effects of Left Prefrontal rTMS on Laboratory, Neuropathic and Post-Operative Pain

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Sponsor: Mark George

Background: Chronic pain is a large public health concern affecting millions of Americans and resulting in billions of dollars per year in direct and indirect healthcare costs, lost-wages and disability expenses. Pain is a complex experience that has sensory-discriminatory, motivational-affective and cognitive-evaluative dimensions and depression prevalence rates in patients with persistent pain range from 30%-54% when rigorous criteria are used to diagnose depression. Chronic motor cortex stimulation (MCS) via implanted electrodes has been used to achieve pain control in patients with intractable neuropathic pain. However, the mechanisms by which MCS controls pain are unclear. Some research suggests that MCS is associated with greater increases in anterior cingulate activity than increases in thalamic or brainstem activity, suggesting that MCS may work by impacting the affective component of pain experience. Unfortunately, MCS is an invasive and expensive surgical procedure. There is emerging evidence that repetitive Transcranial Magnetic Stimulation (rTMS; which is non-invasive and relatively inexpensive) can alleviate the experience of acute and chronic pain (especially neuropathic conditions). The majority of TMS-pain studies to date have investigated the effects of rTMS applied over motor cortex on neuropathic pain, with some promising results. However, if indeed MCS works by impacting the affective dimension of pain, it is possible that rTMS may be optimized for pain if it is used to stimulate the left prefrontal cortex (which has been shown to result in significant limbic activity including the anterior cingulate, and is associated with improvement in depression).
Methods: In the current studies, we are investigating the effects of left prefrontal rTMS (100% of resting motor threshold, 10 Hz, 10-second stimulus train, 20-second interstimulus interval for 20 minutes; a total of 4000 pulses) on controlled laboratory pain (thermal and mechanical), neuropathic pain (measured by pain diaries), post-operative pain (measured by Patient Controlled Analgesia (PCA) pump usage in gastric bypass surgery patients), and changes in mood.
Results: Preliminary results suggest antinociceptive trends associated with prefrontal rTMS in laboratory-induced (N=7) and post-operative pain (N=8). It is expected that by December of 2005, data will be available on 12 healthy adults, 10 neuropathic pain patients and 20 post gastric bypass surgery patients.
Discussion: rTMS may have promise as a treatment for certain chronic pain conditions (especially neuropathic pain and in patients with co-morbid depression). More controlled systematic research is needed to help determine the reliability of the observed preliminary antinociceptive effects and to help clarify mechanisms of action.

139. Indiplon Dose-Response Analyses For Objective and Subjective Measures of Sleep

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Sponsor: Dimitri Grigoriadis

Background: Indiplon is a selective GABA-A receptor potentiator under investigation for the treatment of insomnia. The purpose of these analyses was to describe the dose-response (DR) relationship of indiplon for polysomnography-derived (PSG) measures of sleep initiation (latency to persistent sleep or LPS), total sleep time (TST), as well as subjective measures of sleep maintenance (subjective total sleep time or sTST and subjective wake after sleep onset or sWASO). The impact of baseline, formulation (IR capsule, MR tablet), gender, time, and age (elderly, non-elderly adults) was also evaluated.
Methods: Data from four crossover Phase 2 and two parallel Phase 3 PSG studies were pooled for the PSG-derived analyses. Data from four crossover Phase 2 and five parallel Phase 3 outpatient studies were pooled for the subjective measures derived analyses. Doses ranged from 5 to 40 mg. Study duration ranged from 2 days to 3 months. DR for each parameter was modeled using a nonlinear mixed effects modeling approach (NONMEM Version V, UCSF). Predictive checks (PC) were performed for model validity by simulating data for 100 replicate studies using the final model and comparing results to observed data.
Results: 773 patients (5940 observations) were included for the PSG analyses, with a mean age of 59 years, 65% women, and 39% receiving the IR formulation. 2492 patients (109,940 observations) were included for subjective measures (sTST, sWASO). Mean age in the subjective measures dataset was 55 years, with 53% receiving IR, and 63% being female. For sleep initiation, indiplon demonstrated an asymptotic reduction from baseline LPS with a maximal reduction of 72% (Emax), with half that value achieved with a dose of 7.7 mg indiplon (ED_{50}). Reduction in LPS increased with age. Placebo response averaged 12%. For TST, baseline values decreased with age (1.08 min per year). TST response with indiplon increased linearly with dose and was steeper in patients with low baseline values. For sTST, baseline was dependent on patient gender, with females having slightly longer baseline sTST (2.86%), study grouping (patients in MR studies had more severe subjective insomnia), and patient age. The DR relationship for sTST was characterized with a maximum value of 386 minutes and ED_{50} of 7.76 mg. The sTST response with the MR formulation was larger (by 11.4%) than with IR. For sWASO, baseline values increased with age. Baseline sWASO was also dependent on gender, with females having slightly higher baseline values (by 5.38%). The sWASO DR relationship was asymptotic with an ED_{50} of 16.6 mg (a 15 mg dose in a typical adult patient yields a 26 minute decrease in sWASO). Magnitude of response was larger in patients receiving the MR formulation (by 39.6%), in females relative to males, and in patients enrolled in sleep lab studies. The response observed in patients receiving indiplon was sustained over time. PC showed that the models adequately described the response by dose and covariates for each model.
Discussion: These analyses suggest that the dose of indiplon required depends on sleep complaint as well as population. For sleep initiation, near maximal effect (i.e., Emax) on LPS is achieved at 10 mg IR in adults. For sleep maintenance, increased benefit is observed at doses higher than 10 mg. For sleep maintenance, The MR formulation provides increased benefit over a similar dose of the IR formulation. Elderly differ in both disease severity and sensitivity, and less drug may be required in this population.

140. Plasma L-Tryptophan Depletion and Loading Time-Course and Side-Effect Comparison of 50 and 100g Formulations

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Sponsor: John Overall

Background: Acute L-tryptophan (Trp) depletion (T-) or loading (T+) is a popular method for manipulating CNS serotonin (5-HT) synthesis to investigate causal biological mechanisms of psychiatric disorders, especially disorders involving impulsive or aggressive symptoms. However, variations in amino-acid dosages across studies,

limited plasma time-course evaluation, and high attrition due to side effects have proven problematic.
Methods: To provide solutions to these problems, the current study compared the effects of 50 and 100g beverages on mood and bodily symptoms, and provides an 8-hr time-course comparison of plasma free and total Trp, along with 5 large neutral amino acids (LNAAs; isoleucine, leucine, phenylalanine, tyrosine, and valine) that compete with Trp for transport across the blood-brain barrier. The commonly used 100g T- (0.0g Trp) and T+ (10.30g Trp) formulations were compared to three 50g amino acid beverages: T- (0.0g Trp), T+ (5.15g Trp), and balanced (1.15g Trp). A total of 120 men and women each consumed one of the five amino acid formulations at 9:30 AM. Plasma samples were collected hourly across an 8-hr period beginning with baseline samples at 8:30 AM and post-drink samples at 10:30, 11:30, 12:30, 1:30, 2:30, 3:30, and 4:30. Participants rated mood and bodily symptoms on a visual-analog scale at baseline and again at peak plasma effect of the amino acid beverages. We assessed the complete time course of (1) free plasma [Trp], (2) total plasma [Trp], (3) the [Trp]/[LNAA] ratios, and concentrations of (4) non-esterified fatty acids, and (5) albumin, along with the self-ratings of mood and bodily symptoms.
Results: Compared to the 100g doses, the 50g doses: (1) achieved similar circulating [Trp] and [Trp]/[CAA] ratio changes; (2) produced fewer negative mood and bodily symptoms; and (3) resulted in less participant attrition.
Discussion: Collectively, these results support the use of the 50g, instead of the larger 100g dose, since comparable plasma changes can be achieved without the loss of data due to side effects and attrition.

141. Prevalence of Intact Decision-Making Capacity for Research Among People with Schizophrenia Using Two Standards

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Sponsor: Dilip Jeste

Background: Ongoing debate surrounds the decision-making capacity (DMC) of people with schizophrenia to consent to research. Much empirical work has been conducted using structured capacity measures, the MacArthur Competence Assessment Tool for Clinical Research (MacCAT-CR) being the most widely used. Yet, no clear guidelines exist for interpreting the results obtained. Various algorithms have been used, including the NIMH CATIE Study (requiring ≥ 15 on the MacCAT-CR Understanding subscale); this cutpoint has now also appeared in other studies.[1] Other criteria, using normative values to set thresholds, have also been explored.[2] Because substantial variation in classification could result from different criteria, we examined the prevalence of capacity associated with two previously published standards, exploring the characteristics associated with capacity classification under each. We hypothesized that the multidimensional criterion (see Kim et al.,[2] who used normative standards on three main domains of DMC) would lead to a lower prevalence of capacity compared to the less stringent criterion used in CATIE. We predicted that cognitive factors would be more consistently associated with capacity than psychopathological variables.
Methods: 91 individuals with schizophrenia or schizoaffective disorder, all ≥ 50 years of age, participated. After receiving information regarding a hypothetical, placebo-controlled randomized clinical trial, all participants were administered the MacCAT-CR, a standardized instrument designed to assess four domains of DMC: understanding, appreciation, reasoning, and choice. Levels of psychopathology, cognitive functioning, and insight were also assessed. Based on the MacCAT-CR results, participants were categorized as having impaired or intact capacity based on 2 standards: (1) the CATIE Study standard (intact: MacCAT-CR Understanding score ≥ 15), and (2) the multidimensional criterion validated by Kim et al.[2] (intact: MacCAT-CR Understanding ≥ 18, Appreciation ≥ 5, and Reasoning ≥ 6).
Results: The prevalence of intact DMC under the CATIE versus multidimensional standard was 92% vs. 42%, respectively. Thus, 50.5% of the sample (n=46) were categorized as intact by the first criteria but impaired by the latter standard. Of these 46 individuals, 39 were classified as impaired based on subthreshold performance on the MacCAT-CR Appreciation subscale alone (n=9), on the Reasoning subscale alone (n=14), or on both Appreciation and Reasoning (n=16). Demographic factors were not associated with capacity classification under either of the standards. In contrast, negative symptoms, insight, and DRS Memory scores were different among those with impaired vs. intact DMC, regardless of criterion. By the multidimensional standard, those with intact capacity also had significantly better overall cognitive functioning compared to those classified as impaired.
Discussion: Different MacCAT-CR criteria led to substantially different proportions of patients being categorized as decisionally intact. Most participants had very good understanding of the hypothetical trial. Requiring above-threshold performance on measures of appreciation and reasoning accounted for much of the difference in classification rates. These results underscore the need for refinement of capacity assessment procedures and for broader consensus on how decisional capacity status is determined. 1. Dunn LB, Nowrangi M, Palmer BW, Jeste DV, Saks E. Assessing capacity to consent to treatment and research: A review of instruments. *Am J Psychiatry* In press. 2. Kim SY, Caine ED, Currier GW, Leibovici A, Ryan JM. Assessing the competence of persons with Alzheimer's disease in providing informed consent for participation in research. *Am J Psychiatry* 2001;158:712-717.

142. Methamphetamine-Induced Dopamine Complex Formation: A Role For Reactive Oxygen Species and Dopamine Receptors

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Background: Methamphetamine (METH) is a psychostimulant of abuse that, when administered at high doses, leads to dopaminergic deficits persisting weeks to months after treatment. In rats, these persistent deficits are prevented by pretreatment with both D1 and D2 receptor antagonists. In addition, multiple, high-dose administrations of METH cause sodium dodecyl sulfate (SDS)-resistant dopamine (DA) transporter (DAT)-associated complex formation, as assessed 24 - 36 h after treatment. The purpose of the current study was to investigate mechanisms contributing to DAT complex formation.
Methods: Rat striatal tissues were subjected to SDS-polyacrylamide electrophoresis and western blotting.
Results: Results revealed that pre-treatment with D1 or D2 receptor antagonists prevented the METH-induced DAT complex formation. This effect was independent of METH-induced changes in core body temperature. In addition, roles for reactive oxygen and nitrogen species formation were suggested by findings that: 1) in vitro application of hydrogen peroxide or peroxynitrite promoted complex formation; and 2) METH treatment in vivo increased immunoreactivity of the reactive species-associated proteins, manganese superoxide dismutase (MnSOD) and heat shock protein-32 (HSP-32), as assessed 24 and 36 h following administration.
Discussion: The implications of these findings with regard to METH-induced dopaminergic deficits, as well as neurodegenerative disorders, will be discussed (supported by DA00689, DA04222, DA11367, DA11389, DA019447 and DA00378).

143. Response to Methylphenidate in Symptoms of Most Concern to Parents of Preschool Children with Developmental Disorders

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Sponsor: James Harris

Background: Assessing treatment response in psychopharmacological trials via standardized scales has many advantages, but may fail to

capture change in behaviors most concerning to an individual family. In a methylphenidate efficacy and safety study to treat hyperactivity, impulsivity and/or distractibility in preschool children with developmental disorders, we addressed parents' specific concerns regarding their child's behavior by including an individualized target symptom assessment in addition to the standardized scales. The primary objective of this poster presentation is to report preliminary data regarding target symptom outcome during the open-label titration phase of the study. We hypothesized that methylphenidate treatment would show improvement in target symptoms at the end of titration compared to the baseline. This NIMH sponsored study was approved by the University of Arizona Institutional Review board.

Methods: Ten boys (mean age=62 + 9 months) with a diagnosis of pervasive developmental disorder confirmed by the Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Scale or with a diagnosis of mental retardation confirmed by the Differential Abilities Scale, and problems with hyperactivity, impulsivity and/or distractibility confirmed by a T-score of 65 on the Hyperactivity-Impulsivity subscale of the Conners Parent and/or Teacher Rating Scale-Revised have completed the methylphenidate and placebo crossover study. Each child undergoes a pre-treatment baseline visit, scheduled at the end of a single-blind placebo lead-in phase, followed by open-label methylphenidate dose titration and double blind methylphenidate and placebo crossover phases. At screen, caregivers identified 2 problems of greatest concern to them; behavioral description and quantification of the 2 target symptoms were recorded at screen, baseline and end of open-label titration. Additionally, a 7-point clinical global impression of severity and improvement (CGI-S and CGI-I) was recorded. After all data were collected, the 20 target symptom descriptions were coded by 3 raters on a 9-point scale, with a score of 5 indicating no change, < 5 indicating improvement and > 5 indicating worsening. To evaluate any rapid placebo response, target symptoms at baseline were coded in reference to the screen visit. The end of titration visits were coded in reference to the baseline visits.

Results: The intra-class correlation among the raters was .88 for baseline and end-of-titration ratings. The end of open-label titration target symptom summary ratings (averaged across the 2 target symptoms in each child) correlated with the end-of-titration CGI-S ($r = .75$, $p = .011$) and CGI-I ($r = .65$, $p = .04$) ratings. ADHD-related behaviors were the most often cited target symptoms (11 of 20), followed by aggression/tantrums (5) and stereotypy (4). The mean baseline and end of open-label titration ratings were 4.48 + .56 and 2.82 + .95 respectively for the 2 target symptoms combined, 4.73 + .68 and 2.91 + 1.05 for the ADHD-related behaviors, 4.34 + 1.27 and 2.92 + .9 for the aggression/tantrums category, and 4 + .55 and 2.1 + .79 for the stereotypy category. The mean end of open-label titration ratings showed significant improvement over the null hypothesis of no change, all ps <.005. We have not yet analyzed primary efficacy and safety data for the double-blind phase since the study is ongoing and the blind has not been broken.

Discussion: Early review of the data collected thus far suggests that target-symptom assessment can be a reliable, valid, and useful method to assess requested behavior changes by parents of preschool children with serious developmental and behavioral problems and can have clinical and scientific utility.

144. Inhibition of Dopamine Transporter Activity by Cannabinoids

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Sponsor: Alan Frazer

Background: Cannabinoids are known to indirectly affect dopaminergic transmission in different brain areas. Although CB_1 receptors are not expressed on dopaminergic neurons, CB_1 agonists increase dopamine (DA) efflux in rat nucleus accumbens by reducing GABAergic input to VTA dopaminergic neurons. In this study we used in vitro and in vivo approaches to investigate whether cannabinoid drugs may affect dopaminergic transmission in mouse striatum via an alternative mechanism, such as modulation of dopamine transporter (DAT) activity.

Methods: Animals. 8-10 week old, male C57BL/6 mice were individually housed with ad libitum access to food and water on a 12 h light-dark cycle. **[^{3}H]DA uptake assay**. Dorsal striata were dissected and processed as described by Moron et al. (2003). Synaptosomes (P2 pellet) were preincubated with WIN 55212-2 (WIN), AM251, or S(-)WIN 55212-3 for 15 min at 37 °C. After preincubation, samples were placed on ice and 0.1 μM [^{3}H]DA was added to initiate uptake (at 37 °C for 4 min). Uptake was terminated by placing samples on ice. Synaptosomal suspensions were layered onto nitrocellulose membranes pre-soaked in 0.1% polyethyleneimine to reduce non-specific binding. Filters were washed 3 times with ice-cold Krebs-Ringer buffer and placed into scintillation vials to count radioactivity. **Binding studies**. Displacement of [^{3}H] WIN 35,428 binding in striatal tissue was performed according to Coffey and Reith (1994). **In vivo chronoamperometry**. High-speed chronoamperometry was carried out according to Daws et al. (2002; 2005). Briefly, Nafion-coated carbon fiber electrodes calibrated for DA in vitro were attached to a multibarrel pipette and lowered into the dorsal striatum of anesthesized (chloralose 85 mg/kg and urethane 850 mg/kg, i.p.) mice. Stereotaxic coordinates were AP+1.1; ML+/- 1.4; DV -2.25. Multi-barrel micropipettes were filled with DA (200 μM, pH 7.4) and DA was pressure-ejected at 5 min intervals until a reproducible baseline was established. Drugs or vehicle were applied by intraperitoneal injection. Changes in peak DA signal amplitude and T80, (the time it takes for the DA signal to decline by 80% of its peak amplitude) were used to assess the influence of drug on DA clearance.

Results: Incubation of striatal synaptosomes with the cannabinoid agonist WIN significantly decreased DA uptake in a dose-dependent manner (IC_{50} 0.75 μM). WIN produced a 39.9% inhibition of DA uptake at the concentration of 1.0 μM. A similar inhibitory effect was observed after application of S(-)WIN 55212-3 (IC_{50} 2.8 μM), which lacks pharmacological activity at the CB_1 receptor. The CB_1 antagonist AM251 did not reverse the inhibitory effect of WIN and produced a significant inhibition of DA uptake (IC_{50} 0.61 μM), when applied alone. The DAT inhibitors GBR and nomifensine displaced the binding of the cocaine analog [^{3}H]-WIN 35,428 with similar potency (IC_{50} of 18 nM and 17 nM, respectively), whereas neither WIN nor AM251 acted as competitive inhibitors (IC_{50} 8.6 μM and 48.4 μM, respectively), suggesting that these drugs do not serve as DAT substrates. In vivo chronoamperometry measurements showed that WIN (4 mg/kg, i.p.) produced a significant release of endogenous DA followed by a transient increase of the T80. AM251 (1 and 4 mg/kg, i.p.) increased the signal amplitude and reduced the clearance of pressure-ejected DA in the striatum dose-dependently. This effect persisted over 40 min at the dose of 4mg/kg.

Discussion: These data indicate that cannabinoid drugs increase dopaminergic transmission in mouse striatum by inhibiting DAT activity via molecular targets other than CB_1 receptors. Supported by NS050401 (AG); DA018992 (LCD)

145. Differences in Cognitive Function and Acute Sedative Effects of Risperidone and Quetiapine Among Individuals with Stable Bipolar I Disorder

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Sponsor: Harbans Lal

Background: Medication-induced sedation is manifested by drowsiness, slowed brain activity, reduced wakefulness, and impaired cognitive performance.1 Because individuals with bipolar I disorder (BID)

are often engaged in competitive employment, these impairments are problematic due to their potential for causing disruption in social-vocational pursuits. The primary objective of a recently completed study was to compare risperidone and quetiapine in their treatment effects on cognitive function in individuals with stable BID, using measures commonly believed to be affected by sedation. Secondarily, the study was to compare treatment effects on the subjective experience of sedation and to assess the association between subjective experience of sedation and cognitive function.
Methods: A randomized, double blind, 2x2 crossover study was conducted at a single center. One-half of subjects were randomized to treatment sequence risperidone-quetiapine (R-Q), and the other one-half to quetiapine-risperidone (Q-R). Subjects received 2mg of risperidone with dinner, or 100mg of quetiapine with dinner and 100mg with breakfast, at each of two 2-day treatment periods. Separating treatment periods was a 6- to 14-day washout period. A computerized battery of eight tests of cognitive function, including three Continuous Performance Tests (AX, IP, and Flanker), Auditory Digit Span, Auditory Number Sequencing, Strategic Target Detection, Word List Memory, Symbol Digit Substitution, and the Visual Analogue Scale for Fatigue2 were assessed. Prior to dosing, baseline assessments were taken on day 1 of each treatment period; post-treatment assessments were repeated three times throughout the day on day 2 of each treatment period. Subjects were aged 18-55 years, with a DSM-IV diagnosis of BID in partial or full remission, and a Young Mania Rating Scale score ? 8 at screening.
Results: Only preliminary findings are currently available. Thirty subjects were randomized; 28 subjects took all doses of study medication, and completed a baseline cognitive assessment battery and at least one post-baseline assessment in each treatment period (the evaluable population). Reasons for discontinuation included withdrawal of consent (n=1, R-Q), and non-compliance with the study protocol (n=1, Q-R). The evaluable population was 40.9 (SD=7.42) years of age on average, 71.4% male, 60.7% black, and had 10.0 (SD=6.85) years since diagnosis on average. Concomitant medications most frequently taken by subjects (N=30) included lithium (10%), antidepressants (10%), and anti-epileptics (10%). The most common adverse events reported during the treatment were somnolence (31%, 82.8%), fatigue (13.8%, 20.7%), and dry mouth (0%, 10.3%) during the risperidone and quetiapine treatment periods, respectively. No serious adverse events were reported during the trial.
Discussion: Complete study findings on the treatment effects on cognitive function, subjective experience of sedation, and the association between subjective experience of sedation and cognitive function will be reported.

146. Family Study of Binge Eating Disorder

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Sponsor: Ming Tsuang

Background: Binge eating disorder (BED) is a common condition that is associated with obesity. However, it remains unclear whether BED represents simply a non-specific eating pattern often seen in obese individuals, or represents a condition that is etiologically distinct from other obesity phenotypes.
Methods: We conducted a blinded family interview study of 300 overweight or obese probands with and without BED, and 888 of their first-degree relatives. We assessed whether BED aggregates in families, and whether BED coaggregates with other eating disorders or with major mood disorders.
Results: BED aggregated strongly in families independently of obesity (odds ratio, 2.2; P<0.001). BED showed little coaggregation with anorexia nervosa, bulimia nervosa, or major depressive disorder, although our ability to detect coaggregation in the latter case may have been diminished by our restriction to overweight or obese probands. However, BED coaggregated significantly with bipolar disorder (odds ratio, 1.8; P=0.008).
Discussion: BED is a familial disorder, attributable to factors at least partly independent of those for obesity, yet partly shared with those for bipolar disorder. Combining this evidence with prior evidence from twin studies, it appears that some of these factors are genetic.

147. Duloxetine: Summary of Time to Response in a Preclinical and a Clinical Model of Neuropathic Pain

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Background: Duloxetine is a relatively balanced, potent and selective reuptake inhibitor of serotonin (5-HT) and norepinephrine (NE) that lacks significant affinity for other neuronal receptors or ion channels. Both 5-HT and NE, in addition to their role in the pathophysiology of depression, have been implicated as modulators of endogenous analgesic mechanisms in descending inhibitory pain pathways in the brain and spinal cord. Animal models of pain behavior have demonstrated the potential benefits of dual 5-HT/NE reuptake inhibition when compared with action upon either 5-HT or NE alone (Iyengar et al, J Pharmacol Exp Ther, 2004: 311). We studied the time to onset of reduction in pain with duloxetine pre-clinically in a model of neuropathic pain, and clinically in patients with neuropathic pain due to diabetic peripheral neuropathy. Duloxetine has been approved by FDA and EU for the treatment of major depressive disorder, as well as the management of diabetic peripheral neuropathic pain (DPNP).
Methods: Preclinical - Male Sprague-Dawley (SD) rats were evaluated for effects of duloxetine (10-30 mg/kg, po) on mechanical allodynia behavior (withdrawal threshold to von Frey filaments) in L5/L6 spinal nerve ligated rats. Clinical - Three placebo-controlled, double-blind studies of nondepressed patients with DPNP ≥ 6 months were conducted. In study 1 (N=457), patients with DPNP were randomly assigned to treatment with duloxetine 20 mg QD, 60 mg QD, 60 mg BID, or placebo. In studies 2 (N=334) and 3 (N=348), patients with DPNP were randomly assigned to treatment with duloxetine 60 mg QD, 60 mg BID, or placebo. In all three studies the weekly mean score of 24-hour average pain severity on the 11-point Likert scale was evaluated as the primary outcome measure for treatment effect, and post-hoc analysis on diary data from the first 6 days was also performed.
Results: Preclinical: In the L5/L6 spinal nerve ligation model, duloxetine reversed mechanical allodynia behavior after a single treatment and retained efficacy after 4 day sub-chronic dosing. Clinical - At 1 week, in all three studies, patients treated with duloxetine 60 mg QD or 60 mg BID had significantly less pain than patients treated with placebo. Daily diary data analysis revealed that duloxetine 60 mg QD significantly reduced pain compared to placebo by days 1, 2, and 4 of therapy in the three studies respectively. At least 50% of duloxetine 60 mg BID treated patients achieved response (30% reduction in 24-hour average pain severity) at Week 2 for two of the three studies, and at Week 3 for the other. For patients treated with 60 mg QD, that observation was seen at Week 2, and 4, respectively.
Discussion: Duloxetine causes rapid reversal of pain behavior in a preclinical model of neuropathic pain and rapid reduction of pain symptoms in clinical studies of diabetic peripheral neuropathic pain.

148. Seizure Risk Among Patients Participating in Psychopharmacology Clinical Trials

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Sponsor: Walter Brown

Background: There is longstanding concern that exposure to most psychotropics increases seizure among patients without history of seizures or that these agents increase the frequency and intensity of seizures among patients with epilepsy. With the exception of a few of the newer psychotropics such as clozapine and bupropion, the data suggesting increased seizure risk is scant. Paradoxically, some recent publications suggest that many of newer antidepressants may decrease seizure rates among patients with epilepsy. In order to asses the seizure risk among psychiatric patients, without a known history of seizure disorder exposed to a variety of psychotropics, we conducted the present study. We considered that psychopharmacology clinical trials, especially those that include a placebo arm would provide data to asses both seizure risk among psychiatric patients as well as allow us to assess any change in seizure with psychotropics.
Methods: We obtained our data from the FDA summary Basis of Approval Reports (SBA) from Phase II and III clinical trials. We surveyed data for antidepressant, antipsychotic, and anti-anxiety drugs approved in the United States between 1985 and 2004, involving a total of 70, 465 patients. We examined differences between the incidence of seizures in the active drug and placebo treated groups using Pearson chi-square statistic among the three drug indication categories.
Results: Overall, the data suggests that patients assigned to placebo had a rate of seizures based patient exposure years (PEY) thirteen times greater than the unprovoked seizure rate reported in the general community (767/100,000/yr compared to 56/100,000/yr). The seizure risk was highest among patients participating in antipsychotic trials (0.8%) followed by patients participating in anxiolytic trials (0.8%) and lowest risk was seen among patients participating in antidepressant trials (0.4%). Much of this risk was associated with psychotropics known to increase seizure risk; clozapine, clomipramine, bupropion and alprazolam (withdrawal seizures). Using PEY data, excluding the data for high seizure risk antipsychotics, the frequency of seizures among patients exposed to antipsychotics (1,150/100,000/yr) was not significantly different from frequency of seizures among patients exposed to placebo (784/100,000/yr). PEY data was not available among anxiolytic trials. Using the PEY data, excluding data from high seizure risk antidepressants, the frequency of seizures among patients exposed to antidepressants (535/100,000/yr) was significantly lower than the frequency of seizures among those patients exposed to placebo (1,502/100, 000,/yr, ?2=4.76, df=1, p=0.029).
Discussion: These data are similar to product labeling information in the PDR, although not widely accepted for clinical practice. Further, these data suggest that a few psychotropics should be avoided whenever possible among patients with history of seizure disorder or increased risk for seizures. However, a majority of psychotropics have little effect on new onset of seizures and most interestingly newer antidepressants may decrease seizure risk.

149. Early Life Stress Impairs Memory and Produces Hippocampal Degeneration in Adult Rats

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Sponsor: Thomas Kosten

Background: Early life events have profound consequences for the organism. Our research demonstrates that the early life stress of neonatal isolation in rats leads to immediate and enduring neural and behavioral sequelae. Recently, we found that neonatal isolation impaired hippocampal-dependent context conditioned fear in adult male rats. We now expand upon this finding to test whether neonatal isolation impairs performance in inhibitory avoidance and in the non-aversive hippocampal-dependent object recognition task. Because we predict that neonatal isolation will impair memory, we also examined whether neonatal isolation results in cellular compromise in the adult hippocampus.
Methods: Litters born to Sprague-Dawley dams were assigned to neonatal isolation (1-h individual isolation on postnatal days 2-9) or control conditions as in our previous research. As adults, (PN60-90), rats were assigned to one of three experiments to test memory performance or hippocampal pathogenesis. Experiment 1 (9-10/group) tested inhibitory avoidance, a task that assesses memory by measuring the latency to enter a dark compartment paired 48-h earlier with footshock. Experiment 2 (11-18/group) tested object recognition, a non-aversive task that exploits the natural tendency of rats to explore novel objects. Separate groups were tested after a 4-h or a 24-h delay. Experiment 3 (11-12/group) investigated hippocampal pathogenesis by measuring antibodies that selectively label calpain-mediated spectrin breakdown product (BDP), a well-established marker of early cytoskeletal damage and cellular vulnerability. Calpain-mediated cytoskeletal damage is frequently associated with synaptic decline. Thus, tissue sections were also stained for the postsynaptic protein GluR1, an AMPA receptor subunit abundantly found in hippocampus.
Results: Neonatal isolation impaired inhibitory avoidance memory ($P<0.005$) and object recognition memory ($P<0.05$), particularly after the 24-h delay, in both male and female rats. Hippocampi from isolated rats exhibited a marked increase in BDP labeling in pyramidal neurons and adjoining neuropil indictative of cellular degeneration. Staining in proximal dendrites was often punctate in nature compared to control rats. Interestingly, the same hippocampal subfields exhibited reduced staining for the postsynaptic marker, GluR1. Normal GluR1 labeling was seen in control tissue where it was densely distributed throughout the stratum radiatum and stratum oriens and sparsely found in neuronal cell bodies. As found in previous studies, BDP production was associated with decreased levels of the postsynaptic marker GluR1, suggesting synaptic decline. The correlation between BDP production and GluR1 decline was significant ($r= -0.50$; $P=0.035$).
Discussion: Neonatal isolation may render those neurons involved in memory encoding to be vulnerable to calpain dysregulation and synaptic decline as shown previously with brain injury. Together with our prior research showing enhanced striatal-dependent learning and neurochemical responsivity, these results indicate that the early experience of neonatal isolation causes enduring yet opposing region-specific neural and behavioral alterations.

150. The Role Of Postsynaptic Alpha-2 Noradrenergic Receptors In The Modulation Of Cortical Activity

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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: In the prefrontal cortex, alpha-2 noradrenergic (α2 NER) receptors function as presynaptic autoreceptors, as heteroreceptors for other modulators, and as postsynaptic receptors. Several studies have shown that adrenergic agonists, especially specific α-2 agonists, are very effective in enhancing working memory (WM) and attention. Indeed, administration of α-2 agonists can ameliorate some of the negative effects on cognition produced by NE depletion due to aging in monkeys (Arnsten and Goldman-Rakic, 1985; Arnsten et al., 1988; Arnsten and Leslie, 1991) and improve performance in WM-related tasks in young monkeys with NE depletion (Arnsten and Goldman-Rakic, 1985; Cai et al., 1993). Moreover, the therapeutic effects of the specific α-2 agonists, clonidine and guanfacine in treating disorders related to dysfunction of WM in patients have been

proved (Fields et al., 1988; Mair and McEntree 1986, 1988; Hunt et al., 1985, 1990, 1995; Horrigan and Branhill, 1995). However, the cellular mechanisms underlying the beneficial effects of α- 2 NER agonist are unknown.
Methods: Whole-cell clamp recordings in current and voltage clamp mode were performed in pyramidal cells located in deep layers of the infralimbic and prelimbic cortices in rat slices. Using IRDC illumination cells were identified and patched. Drugs and channel blockers were bath-applied. Intrinsic and synaptic stimulation were evaluated, as well as basic electrophysiological characteristics.
Results: We have shown (Andrews et al, Soc, Neuroscience, 2005) that activation of α-2 NER by the α-2 agonist clonidine (10 μM) increases intrinsic excitability in deep layer pyramidal neurons recorded in slices, however, clonidine can also activate α-1 receptors. In order to assess the specificity of our results, we applied the specific α-1 antagonist prazosin (1μM) prior clonidine administration. It was found that clonidine still increased pyramidal cell activity. This observation is intriguing given that α-2 receptors are Gi coupled, which would lead one to hypothesize an inhibitory effect. We seek to reconcile the observed results from those expected. Thus we propose the following hypothesis: The increase in pyramidal excitability following activation of α-2 receptors is an indirect effect mediated by a disinhibition of interneurons. In order to test the hypothesis we analyzed the effects of clonidine in the presence of NMDA (CPP 10 μM), non-NMDA (CNQX 10 μM) and GABAA (bicuculline, 10 μM) blockers. In those conditions the α-2 agonist did not elicit increases in pyramidal excitability, yet when only glutamatergic blockers were present (CNQX 10 μM and CPP 10 μM) clonidine increased pyramidal excitability. Voltage-clamp experiments in presence of CNQX and CPP revealed that clonidine evoked a decrease in sIPSC frequency by 36% suggesting a strong presynaptic modualtion (n=6). Our results support the hypothesis that α-2 NER are mediating increases in cortical activity through a decrease in GABA release.
Discussion: We propose that through modulation of cortical networks α-2 agonists improve WM and attention.

151. Effects of Childhood Trauma and Acute Lorazepam on Fear-Related Cortico-Limbic Brain Reactivity: A Pilot Pharmacological fMRI Study

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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: In rodents, level of maternal care modulates frontal and limbic benzodiazepine-sensitive GABA receptor expression. Previous human studies have found that lorazepam acutely causes decreased BOLD response in amygdala and orbitofrontal cortex during viewing of emotional images. It was hypothesized that: 1. Personality disorder subjects with high vs. low levels of self-report childhood trauma would exhibit greater amygdala BOLD reactivity to fearful vs. neutral facial expressions, 2. High vs. low level of self-report childhood trauma would be associated with blunted benzodiazepine deactivation of amygdala BOLD contrast of fearful vs. neutral facial expressions.
Methods: 8 personality disordered (PD) and 5 normal control (NC), right handed, male and female subjects have been studied to date. Level of self-report childhood trauma was measured with the Childhood Trauma Questionnaire (CTQ). All subjects were medication free and without current major mood disorder, psychotic disorder, substance dependence, or PTSD. Functional magnetic resonance imaging (fMRI) at 3Tesla (reverse spiral: TR=2s; TE=25ms) was utilized during viewing of black and white photographs of Ekman emotional faces. In a double-blind, placebo-controlled, randomized design, subjects underwent imaging over 2 sessions (at least 1 week apart), given either 0.5 mg lorazepam or placebo intravenously 20 minutes before the fMRI experiment. During fMRI, subjects viewed 4 runs of alternating 20-sec blocks of Ekman Faces expressing discrete emotions (anger, fear, disgust, happy, sad, neutral), interleaved with 20-sec blocks of gray screens, lasting for 18 minutes, in counterbalanced order. Image analysis utilizing a standard, random effects model to determine emotion, drug, and group effects was performed with Statistical Parametric Mapping software (SPM2), using small volume correction (SVC), threshholded atp < .05.
Results: On placebo, PD compared to NC subjects had significantly greater BOLD contrast to fearful > neutral facial expressions in the right amygdala ([-26, 0 , -16], t = 3.64, SVC). Within the PD group, greater BOLD contrast to fearful > neutral facial expressions in the right amygdala on the placebo session was found in the high vs. low CTQ subgroups ([-26, -2, -14], t = 6.70, SVC). These contrasts appeared to be specific to fearful > neutral contrasts compared to disgust and anger contrasts with neutral faces. Greater right amygdala fearful > neutral facial expressions BOLD contrast in high vs. low CTQ PD subjects remained on the lorazepam challenge day ([-18, -2, -12], t = 6.76, SVC). While the low CTQ PD group showed a placebo > drug difference in right amygdala activation to fearful > neutral contrast ([-16, -8, -22], t = 7.34, SVC), the high CTQ group did not show difference in BOLD activation. Further evidence consistent with reduced effect of benzodiazepine in high CTQ subjects on right amygdala BOLD reactivity to fearful faces was found testing low > high CTQ, placebo > drug, fearful > neutral contrasts ([-28, -10, -18] t = 3.91, SVC).
Discussion: On placebo, high CTQ PD subjects have greater right amygdala activation to fearful faces compared to low CTQ PD subjects and NCs. Intravenous lorazepam may result in decreased right amygdala activation to fearful faces in the low CTQ PD group, but to a lesser extent in the high CTQ group, consistent with the translational hypothesis, that decreased exposure to maternal care results in decreased benzodiazepine-sensitivity in the amygdala. The data is highly preliminary, given the small number of subjects in each cell. Analyses are planned using data from a further 11 subjects, utilizing region of interest analyses.

152. Trophic Factors and Adrenergic Function

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Background: Epinephrine appears to regulate serotonergic, noradrenergic and dopaminergic centers in the brain, the targets for therapeutic drugs in psychiatric illnesses, through proximal alpha 1B adrenergic receptors. The trophic factor NGF seems to be one critical messenger for therapeutic action. Historical precedence suggests that NGF restricts catecholaminergic cells to the noradrenergic phenotype, so that adrenergic cells exposed to this neurotrophin may down-regulate factors sustaining adrenergic expression. To the contrary, recent evidence indicates that NGF may induce the gene encoding the epinephrine-synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT).
Methods: NGF effects on PNMT promoter-driven transcription were assessed by transient transfections assays using PC12 cells and the PNMT promoter-luciferase reporter gene construct pGL3RP893, deletion and site-directed mutant PNMT promoter-luciferase constructs. Signaling pathways involved in NGF-mediated activation of the PNMT promoter were determined by examining the effects of kinase inhibitors (H-89, PKA; GF109203X, PKC; UO126, ERK MAPK; SB203580, p38MAPK; Wortmanin and LYL294002, IP3 and U73122, phospholipase C) on NGF-induced PNMT promoter activity. Changes in PNMT protein and PNMT transcription factors were measured by ECL-western analysis. Transcription factor-DNA binding interactions were examined by gel mobility shift assays (GMSAs). mRNA was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) or real-time PCR (QPCR).
Results: NGF stimulates PNMT promoter-driven luciferase activity in a dose- and time-dependent manner. Induction is attenuated by

inhibition of the extracellular-signal-regulated-kinase (ERK) mitogen-activated protein kinase (MAPK, ~60%) but not by inhibition of protein kinase A (PKA), protein kinase C, phosphoinositol kinase or p38 MAPK. NGF-sensitive sequences reside within the proximal -392 bp of PNMT promoter based on deletion mutation, a region containing functional Egr-1 (-165 bp) and Sp1 (-48 bp) sites. NGF induces nuclear Egr-1 protein but not Sp1 or the catalytic subunit of PKA (PKA-C). The same nuclear extract shows greater Egr-1 and Sp1/DNA binding complex. Site-directed mutation of Egr-1 or Sp1 elements attenuates NGF activation. Both NGF and another trophic factor, pituitary adenylate cyclase activating polypeptide (PACAP) stimulate expression of fully processed (intronless) and intron-retaining (intron 2) forms of endogenous PNMT mRNA in the PC12 cells but in combination, they only produce intronless mRNA.
Discussion: The neurotrophin NGF can sustain the adrenergic phenotype as demonstrated by the ability of NGF to stimulate PNMT promoter-driven luciferase and endogenous PNMT gene expression. One important signaling pathway in NGF induction is that of ERK. PNMT promoter activation by NGF appears mediated by stimulation of Egr-1 protein and its phosphorylation as well as by phosphorylation of existing Sp1 protein. The trophic factor PACAP also induces PNMT promoter-driven transcription and endogenous PNMT mRNA. While both neurotrophins stimulate expression of fully processed and intron-retaining PNMT mRNA , NGF induces a greater rise in intronless PNMT mRNA, the template for active PNMT enzyme. Interestingly, the combination of NGF and PACAP only produces intronless PNMT mRNA. Thus, NGF can markedly activate the PNMT gene, but post-transcriptional events may limit the extent to which this neurotrophin alters protein translation and production of active enzyme.

153. Functional Outcomes in Adults with Attention-Deficit/Hyperactivity Disorder Following Treatment with Atomoxetine vs. Placebo

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Sponsor: Darryle Schoepp

Background: We previously demonstrated (ACNP 2004 poster #160) functional impairment in employed adults diagnosed with attention-deficit/hyperactivity disorder (ADHD). Here we present results from the double-blind portion of this study comparing atomoxetine (ATX) with placebo (PBO), using the Endicott Work Productivity Scale (EWPS; Endicott & Nee 1997, *Psychopharmacol Bull* 33:13-16) as the primary outcome measure.
Methods: The trial enrolled adult patients (ages 18-49) employed for pay at least 20 hours per week and meeting DSM-IV-TR criteria for both a current diagnosis of adult ADHD and a historical diagnosis of childhood ADHD, as assessed by the Conners' Adult ADHD Diagnostic Interview for DSM-IV, with a CGI-Severity-ADHD score ≥4 at Visits 1 and 2. Patients had to be at their current place of employment for at least 6 months. Assessment measures included the EWPS and the investigator- and self-rated versions of the Conners' Adult ADHD Rating Scales (CAARS-INV and CAARS-self, respectively). Patients were seen monthly for 2 months, then bimonthly for the next 4 months. All received blinded study medication once daily for up to 6 months.
Results: Four hundred ten (410) patients were randomly assigned to receive ATX (40-100 mg, *n*=271) or placebo (*n*=139). ADHD symptomatology was similar to that seen in 2 previous large clinical registration trials (Michelson et al. 2003, *Biol Psychiatry* 53:112-120). However, only 38.4% and 48.9% of ATX- and PBO-treated patients, respectively, completed the entire 6-month, double-blind portion of this study. The study failed to show a separation between ATX and PBO for changes in the EWPS (*P*=.406), the CAARS-INV total (*P*=.412), or the CGI-severity-ADHD (*P*=.173), but showed a significant effect for the CAARS-self-rated total (-11.5±11.3, ATX vs. -9.9±10.7, PBO; *P*=.027). Adverse events reported significantly more frequently with ATX than PBO were similar to those seen in previous ATX studies in adults, such as nausea, dry mouth, fatigue, insomnia, decreased appetite, constipation, erectile dysfunction, urinary hesitancy, and vomiting. Post hoc analyses by age (18-30 yrs, *n*=107; 31-41 yrs, *n*=153; and >41 yrs, *n*=150) showed a statistically significant effect for ATX vs. PBO, but only in the youngest adult cohort, ages 18-30, for the EWPS (-19.4±17.5, ATX vs. -10.4±11.7, PBO; *P*=.010), CAARS-self (-12.8±10.4, ATX vs. -5.8±8.5, PBO; *P*=.001), and CGI-Severity-ADHD (-1.2±1.2, ATX vs. -0.6±0.8, PBO; *P*=.016), with a nonsignificant numerical trend for the CAARS-INV (*P*=.067).
Discussion: In this study, a large placebo response and high discontinuation rates appeared to be responsible for the failure to achieve the original objectives. In addition to adverse events, infrequency of visits (using a more naturalistic approach), as well as less rigorous criteria for establishing the presence of ADHD in childhood (required for an adult diagnosis of ADHD) than required in the original adult registration trials, may also have contributed to this failure. The more robust findings noted in the post hoc analyses suggest a potential positive effect for ATX in younger adults. These results may have also resulted from application of less rigorous requirements for validation of childhood ADHD, leading to possible misdiagnosis of older patients. Further work will be necessary to clarify the effect of ATX treatment on work productivity among adults with ADHD and whether an age-related effect exists.

154. Abnormal Glutamate and Dopamine Receptor Function in the Striatum of Alpha-Synuclein Overexpressing Mice

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Background: The presynaptic protein alpha-synuclein has been implicated in several neurodegenerative diseases and synucleinopathies, including Parkinson's disease. Mice overexpressing alpha-synuclein under the Thy-1 promoter (ASO) show abnormal accumulation of alpha-synuclein in cortical and subcortical regions of the brain. To determine how abnormal alpha-synuclein expression affects glutamatergic function, we examined spontaneous excitatory postsynaptic currents (EPSCs) in striatal medium-sized spiny neurons (MSSNs) and cortical pyramidal neurons in slices from ASO mice (90 days) using whole-cell patch voltage-clamp recordings.
Methods: Coronal brain slices containing striatum and cortex were prepared by standard methods. Neurons were visualized using an upright microscope equipped with infrared illumination and differential interference contrast optics. Cells were generally held at different potentials depending upon the purpose of the experiments.
Results: At a holding potential of -70 mV in artificial cerebrospinal fluid (ACSF) in the presence of bicuculline (20 μM, a $GABA_A$ receptor antagonist), the spontaneous EPSC frequency was significantly lower in MSSNs in ASO mice when compared with aged-matched wildtype (WT) littermates. Application of CNQX (10 μM), a non-NMDA receptor antagonist, blocked spontaneous EPSCs, indicating the events were mediated primarily by non-NMDA receptors. Application of TTX (1 μM) significantly reduced the frequency of spontaneous EPSCs in control mice (42%), while producing a smaller reduction in ASO mice (25%), indicating that in ASO mice less spontaneous EPSCs are dependent on action potentials. These effects did not occur in cortical pyramidal neurons. In MSSNs application of amphetamine (25 μM), a dopamine releasing agent and reuptake blocker, significantly reduced the frequency of spontaneous EPSCs in control mice, while producing no effect or even an increase in ASO mice. Sulpiride, a D2 receptor antagonist, tended to increase the frequency of spontaneous events in WTs but decreased it in ASO mice. Quinpirole, a D2 agonist, had opposite effects. In addition to examining spontaneous EPSCs mediated by activation of glutamate receptors, we examined spontaneous inhibitory postsynaptic currents (IPSCs)

mediated by activation of $GABA_A$ receptors in MSSNs by changing the holding potential to +20 mV. Similar to EPSCs, spontaneous IPSCs were also significantly reduced in frequency in ASO mice.
Discussion: Together, these observations suggest that abnormal accumulation of alpha-synuclein alters striatal glutamate receptor function and its modulation by drugs that manipulate the dopamine system and are used to treat Parkinson's disease. These effects appear to be specific to the striatum as they were not present in cortex. Supported by: ES12078, NS38367, and NS33538.

155. Asenapine Preferentially Increases Dopamine (DA) and Acetylcholine (ACh) Efflux in Rat Medial Prefrontal Cortex (mPFC) and Hippocampus (HIP)

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Sponsor: Herbert Meltzer

Background: Asenapine is a novel psychotherapeutic agent with a unique human receptor binding signature showing very high affinity for serotonin, noradrenaline, and dopamine (DA) receptors (Shahid et al; submitted ACNP 2005). In particular, asenapine has very strong serotonergic properties compared with other antipsychotics. Asenapine has shown improved efficacy (negative symptoms and cognition) and good tolerability compared with risperidone in one clinical trial to date in patients with schizophrenia (Potkin et al; submitted ACNP 2005). Preferential increases in DA and acetylcholine (ACh) efflux in the medial prefrontal cortex (mPFC) and hippocampus (HIP) compared with the nucleus accumbens (NAc) have been hypothesized to contribute towards improvement in cognition, negative symptoms, and possibly depression, in patients with schizophrenia.
Methods: In the present study, using in vivo microdialysis in rats, we measured efflux of DA and ACh in the mPFC, HIP, and NAc after acute subcutaneous (sc) treatment with asenapine 0.01, 0.05, 0.1 and 0.5 mg/kg.
Results: We found asenapine 0.05, 0.1, and 0.5, but not 0.01 mg/kg, significantly increased DA efflux in the both the mPFC and HIP. Only asenapine 0.5 mg/kg increased DA efflux in the NAc. As for the effects of asenapine on ACh, acute administration of asenapine 0.1 and 0.5, but not 0.01 or 0.05 mg/kg increased ACh efflux in the mPFC. Only asenapine 0.5 mg/kg increased ACh efflux in the HIP. None of the doses studied here produced increases in ACh efflux in the NAc. The increases in DA and ACh efflux in mPFC and HIP produced by 0.1 mg/kg (sc) asenapine were blocked by the selective 5HT1A antagonist WAY100635 (0.2mg/kg, sc).
Discussion: These results suggest that asenapine produced a novel pattern of neurochemical effects, with a preferential increase in efflux of cortical and hippocampal DA and ACh. Furthermore, at least, part of the mechanism mediating the neurochemical effects of asenapine involves 5HT1A receptor activation. The unique pattern of regional increases in DA and ACh may be of relevance to the effects on cognition and negative symptoms found with asenapine in patients with schizophrenia.

156. Topiramate for the Treatment of Moderate to Severe Binge Eating Disorder Associated with Obesity – A Double-Blind, Placebo-Controlled Study

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Sponsor: Stephen M. Strakowski

Background: Binge eating disorder (BED) is associated with compulsivity, impulsivity and obesity. Topiramate is an antiepileptic with preliminary evidence of effectiveness in BED, other conditions characterized by compulsive and impulsive features (bulimia nervosa, alcohol and cocaine dependence), and obesity. The objective of this study was to evaluate the efficacy and safety of topiramate compared with placebo in the treatment of moderate to severe BED associated with obesity.
Methods: In this 16-wk, multicenter, outpatient, randomized, double-blind, placebo-controlled, parallel-group, flexible-dose (range, 25-400 mg/d) trial, 407 outpatients (18-70 years of age) with DSM-IV BED and obesity (body mass index [BMI]≥ 30 and ≤52 kg/m^2) were randomized to receive topiramate or placebo. Primary efficacy outcome (change in number of binge days/wk) was analyzed using repeated-measures random regression with treatment-by-time as the effect measure. Primary and secondary efficacy measures were also analyzed using an analysis of covariance (ANCOVA; with baseline value as the covariate) in a modified intent-to-treat population (N=394) which excluded subjects with ≥1 of the following inclusion/exclusion violations: no diagnosis of BED, <3 binge days/wk in each wk during the 2-wk baseline period, BMI <30 kg/m^2, and/or Montgomery-Asberg Depression Rating Scale >24. Safety assessments included monitored vital signs, physical examination, clinical laboratory parameters, and adverse events.
Results: Patients receiving topiramate (N=195) showed a significantly greater rate of reduction in binge days/wk than those receiving placebo (N=199) (P<0.001, repeated measures analysis). Reduction in binge days/wk was 72% for topiramate and 47% for placebo (P<0.001, ANCOVA). Topiramate was also associated with significant reductions in binge episodes/wk (topiramate, −73±37%; placebo −47±41% and mean BMI (topiramate, −1.6±1.8 kg/m^2; placebo 0.08±1.2 kg/m^2; both P values <0.001). Remission of binge days was attained by 58% of topiramate-treated patients and 29% of placebo-treated patients (P<0.001). Topiramate was associated with significantly greater improvement in overall, obsessive, and compulsive subscales of the Yale-Brown Obsessive Compulsive Scale (modified for binge eating); overall, motor, and nonplanning impulsiveness scores of the Barratt Impulsiveness Scale, Version 11; cognitive restraint, disinhibition, and hunger subscores of the Three Factor Eating Questionnaire; and overall, social, and family life disability scores of the Sheehan Disability Scale. Median final topiramate daily dose was 300 mg/d (range, 25-400 mg/d). The most common adverse events with topiramate were paresthesia, upper respiratory tract infection, somnolence, nausea, taste perversion, dry mouth, difficulty with concentration/attention, headache, and difficulty with memory NOS. Discontinuations were 30% in both groups with the most common reason for discontinuation in the topiramate group being adverse events (topiramate, 16% and placebo, 8%).
Discussion: In this 16-wk study, topiramate was efficacious and well tolerated in the treatment of moderate to severe BED associated with obesity. It was associated with improvement in binge eating behavior and obesity as well as the compulsive and impulsive features of the condition.

157. Effect of Risperidone and Olanzapine on Measures Associated with the Insulin Resistance Syndrome

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Background: Metabolic side effects of some atypical antipsychotic drugs limit their acceptance by patients and may increase long term risk of cardiovascular disease (CVD). Class labeling of these drugs does not reflect important differences among this class of drugs. We report here the results of the first prospective, randomized 12 months trial of the effects of olanzapine (OLZ) and risperidone (RIS) on weight, body mass index, fasting blood glucose, hemoglobin A1C, triglycerides (TG), cholesterol, and HDL and LDL cholesterol, the TG/HDL ratio, and blood pressure in 147 patients with schizophrenia, schizoaffective disorder, or bipolar disorder.
Methods: Metabolic measures were obtained at baseline, 1, 3, 6 and 12 months. There was a 40 % over all drop out rate by 6 months . Data were analysed with a mixed model repeated measures ANOVA.

This analysis will focus on the 36 patients treated with RIS and 43 patients treated with OLZ who completed 6 months treatment.
Results: Thirty three percent of both patient groups who completed six months showed insulin resistance by conventional criteria and TG/HDL ratio at baseline; the latter signifies a higher rate of development of insulin resistance (IR) and increased risk of CVD. There was a significantly greater effect of OLZ on weight, BMI, HgA1c, total cholesterol, TG , and log TG and TG/HDL ratio; the differences did not become maximal until 6 months treatment. TG levels increased 91 mg/dl in the OLZ group vs 8 mg/dl in the RIS group (p=0.003). TG/HDL ratio increased 2.85 in the OLZ group vs 0.43 in the RIS group(p=0.005). The % of patients with TG/HDL ratio =/>3.5 at 6 months increased to 60.5% for the OLZ group and 47.2% for the RIS groups (chi-square =.1.39, p = 0.2). The increase in lipids and HgA1c was independent of increase in BMI, dose and diagnosis. The effects of OLZ but not RIS were enhanced in some patients treated with some mood stablizers. Patients with preexisting diabetes mellitus tolerated both drugs well. Genetic determinants of vulnerability for increased BMI and lipids by these two antipsychotic drugs were studied and will also be reported.
Discussion: This study suggests that: 1) OLZ produces a greater effect on lipids and HgA1C than does RIS and that comparisons should be made after 6 months treatment; 2) that normal BMI and absence of weight gain during treatment does not preclude lipid abnormalities; 3) that mood stabilizer effects on lipids should be considered in choice of an antipsychotic and 4) that the TG/HDL ratio may be a clinically useful measure of insulin resistance. Supported, in part, by an independent investigator grant to HYM from Janssen Pharmaceutica for the metabolic study and by grants from the Prentiss, Ritter and William K Warren Foundations for the genetic analysis.

158. Multiple Novel Polymorphisms in the Rhesus Macaque DRD4 5' UTR are Associated with Impulsive Behavior

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Sponsor: Charles Bradberry

Background: The human DRD4 gene is replete with sequence variants, including SNPs, a 12 bp deletion in Exon 1, and a functional 48bp repeat polymorphsim in Exon 3 that is associated with a number of behavioral phenotypes, including novelty seeking and attention deficit hyperactivity disorder. The Exon 3 VNTR is present in many non-human primate species, with the exception of rhesus macaques (an important animal model for behavior and pharmacology) where the repeat structure, though present, is invariant. We have sequenced and screened the rhesus DRD4 gene (rhDRD4) for novel variants, and here we present evidence for an association between impulsive behavior and promoter haplotypes.
Methods: We amplified 1.7kb of rhDRD4 sequence, immediately upstream of Exon 1, and sequenced five overlapping, nested primer sets. We used a screening panel of 24 subjects (12 males, 12 females) selected from a pool of 200 assessed for impulsivity using an intruder challenge test. Selection criterion was an extreme measure, in seconds, in their latency to respond to an intruder (i.e., fast or slow).
Results: Based on the 48 chromosomes sequenced to date, we have identified 8 SNPs within the presumed promoter region, and a 32 bp insertion/deletion 273 bp from the putative translation start site. The SNPs form two major haplotypes (A&B), and exhibit high LD (.76) with the insertion/deletion. In subjects with short latency times, the frequency of haplotype B was .66 ($p < .01$) and the frequency of the deletion was .75 ($p < .001$).
Discussion: The rhDRD4 5' UTR shares a number of features with the human DRD4 promoter: It is highly polymorphic (SNPs approximately every 200bp), rich in CpG dinucleotides, contains numerous putative Sp1 sites, and lacks TATA and CAAT boxes. Notably, several of the SNPs occur in Sp1 and CpG sites, and thus may be of functional significance. Our association analysis, while preliminary, suggests a potential role for rhDRD4 in influencing interindividual differences in impulsive behavior, and lends further support to a potential functional role for the reported promoter variants. Our work also emphasizes the value of cross-species comparative analyses of functional gene regions, highlighting potential pharmacological targets.

159. Metabolic Profile in Children and Adolescents with Disruptive Behavior Disorders Treated with Risperidone

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Sponsor: Robert Findling

Background: To determine the relationship between insulin, glucose, and weight gain, in children and adolescents with disruptive behavior disorder (DBD) who responded to 3 months of initial treatment with risperidone and subsequently enrolled in a 6-month, double-blind withdrawal study.
Methods: Subjects (aged 5-17 years) who responded to 12 weeks of acute open-label risperidone treatment enrolled in a 6-month, randomized, double-blind, placebo-controlled, parallel-group multicenter study of risperidone in children with DBD.[1] Laboratory samples (fasting insulin, glucose) were evaluated at 3 time points by descriptive statistics and analyzed by Tanner stage. Changes in body weight, height, and BMI were transformed into z-scores and compared to Centers for Disease Control normative data. Spontaneous reports of metabolic adverse events were also recorded.
Results: Acute risperidone treatment (median dose=0.75 mg/day for subjects <50kg and 1.5 mg/day for subjects ≥50kg) was initiated in 527 subjects. 335 subjects (64%) were classified as sustained responders to risperidone treatment and were randomized in the double-blind phase of the study. Weight z-scores: Weight increased during the 12-week acute open-label risperidone phase. Mean (SD) changes at endpoint were +0.3 (0.27)kg and +0.3 (0.28)kg for placebo (n=163) and risperidone (n=171) subjects, respectively. Subjects who continued risperidone treatment during the double-blind phase showed no additional weight gain above expected, based on age- and sex-matched data [mean change (SD) from double-blind baseline to double-blind endpoint: 0.0 (0.29)kg, n=156]. Placebo subjects' weight decrease [mean change (SD) from double-blind baseline to endpoint: -0.1 (0.22)kg, n=147]. Insulin: Median changes at end point of the 12-week acute open-label risperidone phase were +20 pmol/L and +10.5 pmol/L for subjects randomized to placebo (n=144) and risperidone (n=150), respectively. At double-blind endpoint, median changes from double-blind baseline were -13 pmol/L and -7 pmol/L in placebo (n=132) and risperidone (n=138) groups, respectively. Glucose: Glucose levels remained generally constant during the 12-week acute open-label risperidone phase. Median changes at open-label endpoint were +0.1 mmol/L and +0.15 mmol/L for placebo (n=139) and risperidone (n=146) subjects, respectively. At double-blind endpoint, glucose levels decreased slightly from double-blind baseline in both treatment groups but this was not clinically relevant (median change=-0.1 mmol/L for placebo (n=128) and risperidone (n=135) groups). Triglyceride/Cholesterol: No significant changes in either triglycerides or cholesterol were observed with risperidone treatment during the open-label or double-blind phases. Metabolic adverse events: No correlation was noted between greater than expected weight gain and change in insulin or glucose levels. No glucose-related adverse events were reported throughout the study and no patient developed diabetes (American Diabetes Association criteria).
Discussion: Initial weight increase associated with risperidone treatment stabilized in risperidone-treated subjects during the 6-month double-blind period and was partially reversed for subjects treated

with placebo during this same time period. Weight gain in excess of normal development had no discernible effect on insulin or glucose levels. Supported by Johnson & Johnson Pharmaceutical Research & Development. 1. Reyes M et al. Poster presented at IACAPAP, August 2004, Berlin, Germany.

160. The Association of Fatigue with Poor Virologic Response in Patients Receiving Interferon-Alpha Plus Ribavirin for the Treatment of Hepatitis C

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Background: IFN-alpha plus ribavirin for hepatitis C virus (HCV) is notorious for inducing fatigue, which in turn may influence treatment response. Accordingly, fatigue and other factors relevant to treatment outcome were examined in 98 HCV-infected patients undergoing therapy with pegylated IFN-alpha-2b (PEG IFN) plus ribavirin.
Methods: Patients were recruited from a larger, multi-center randomized trial of fixed dose (800 mg a day) vs. weight-based (800-1400 mg a day) ribavirin in combination with PEG IFN (1.5 mcg/kg/week). Patients were evaluated prior to initiation of PEG IFN/ribavirin and following 4, 8, 12 and 24 weeks of treatment. Concomitant medications and continuing use of PEG IFN and ribavirin were also reviewed. Information on dose reduction of PEG IFN and/or ribavirin was obtained from medical records of treating physicians. The development of fatigue was considered as the maximal fatigue score measured with the Chalder Fatigue Questionnaire (CFQ) at any evaluation point during the first 24 weeks of treatment or the change in CFQ score from baseline to the maximum (delta CFQ). To examine the relationship between varying levels of increased symptoms of fatigue and relevant outcome variables including HCV RNA status after 24 weeks of treatment, increases in CFQ scores (delta CFQ) were categorized into mild (<2 SD increase), moderate (2-3 SD increase) and severe (>3 SD increase) based on the standard deviation (SD) of baseline CFQ scores of the study sample (SD=2.55). To compare differences in fatigue development between patients who did and did not clear virus at 24 weeks, delta CFQ, as well as maximal absolute CFQ score, were also evaluated as continuous variables.
Results: PEG IFN plus ribavirin resulted in profound and persistent fatigue. Sixty-five percent of patients exhibited a moderate to severe increase in fatigue during treatment as defined by a ≥5 point increase in the CFQ. Patients who exhibited a ≥3 g/dl decrease in hemoglobin (Hb) concentration were significantly more likely to experience a moderate or severe increase in fatigue and were significantly more likely to undergo ribavirin dosage reduction. Interestingly, however, although not associated with PEG IFN and/or ribavirin dosage reduction, a moderate or severe increase in fatigue (≥5 point increase in the CFQ) was associated with a greater likelihood of being HCV RNA positive after 24 weeks of treatment, even after controlling for other factors that predicted 24 week HCV RNA status, including viral genotype, PEG IFN and/or ribavirin dosage reduction, and race (adjusted OR, 3.2; 95% CI, 1.2-8.8, $p<0.05$). Patients who were HCV RNA positive after 24 weeks of treatment had significantly greater increases in fatigue during therapy than patients who were HCV RNA negative (delta CFQ for HCV RNA positive=6.8±2.8 vs. 4.9±3.1 for HCV RNA negative, $t=3.11$, $p<0.005$). This represents a 40% greater delta CFQ score in HCV RNA positive patients than in patients who were HCV RNA negative at 24 weeks. HCV RNA positive patients also had a higher mean maximum CFQ score during treatment than patients who cleared virus (8.0±2.0 vs. 6.8±2.9, $t=2.32$, $p<0.05$).
Discussion: In conclusion, fatigue is common during IFN-alpha therapy and is related in part to decreases in Hb concentration. In addition, fatigue appears to have an independent relationship with HCV RNA status at 24 weeks, suggesting a shared pathophysiologic pathway between the development of fatigue and treatment non-response.

161. Randomized, Double-Blind, Placebo-Controlled Study of Armodafinil for the Treatment of Excessive Sleepiness Associated With Chronic Shift Work Sleep Disorder

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Background: Modafinil is a racemic wake-promoting agent containing equal amounts of *R*-modafinil and *S*-modafinil. Armodafinil, the *R*-enantiomer of modafinil, is also a wake-promoting agent that has a half-life approximately 5 times longer than the *S*-enantiomer of the racemic compound (10—15 vs 3 h). The present study assessed the effect of armodafinil on wakefulness and cognitive performance during the night shift in patients with shift work sleep disorder (SWSD).
Methods: This study was a 12-week, randomized, double-blind, placebo-controlled, parallel-group trial conducted at 42 sleep research facilities in North America. A total of 254 permanent or rotating shift workers diagnosed with chronic SWSD who worked at least 5 night shifts/month were enrolled. Patients took study medication only on nights when they worked the night shift or participated in a laboratory night shift. The dose of armodafinil was gradually increased on work nights; patients received a dose of 50 mg on the first night, 100 mg on the second and third nights, and 150 mg on all subsequent nights. During laboratory night shifts, study medication was administered at 2200 hours (±30 minutes). Laboratory night shifts were scheduled at 4, 8, and 12 weeks to assess nighttime sleep latency (Multiple Sleep Latency Test), clinical condition (Clinical Global Impression of Improvement [CGI-I], patient-reported sleepiness (Karolinska Sleepiness Scale [KSS]), and cognitive function (Cognitive Drug Research [CDR] computerized assessment system). Patients also completed electronic diaries related to sleepiness. Safety assessments included clinical laboratory testing, vital sign measurements, electrocardiogram, and monitoring of adverse events.
Results: Patients receiving armodafinil experienced significantly longer sleep latencies during laboratory night shifts than patients receiving placebo (change from baseline at final visit 3.1±4.5 vs 0.4±2.9 minutes, respectively [$P<0.0001$]). CGI-I ratings improved in a greater proportion of patients receiving armodafinil (75% vs 59% for placebo, $P=0.001$), and patient-reported sleepiness was reduced relative to placebo (KSS; $P<0.005$). Armodafinil significantly improved the quality of episodic secondary memory and power and the continuity of attention on the CDR (all $P<0.05$). Electronic diary-derived data also revealed that armodafinil significantly reduced the maximum level of sleepiness during the night shift compared with patients receiving placebo (2.0 vs 1.1 points; $P<0.0001$). Similarly, the level of sleepiness during the commute home was decreased 2-fold for armodafinil relative to placebo (1.2 vs 0.6 points; $P=0.0027$). Armodafinil was well tolerated and did not affect daytime sleep. The most common adverse events were headache, nausea, nasopharyngitis, and anxiety.
Discussion: Armodafinil significantly improved wakefulness, clinical condition, and long-term memory and attention in patients with chronic SWSD without disturbing daytime sleep.

162. A Complementary Strategy to Approach the Genetics of Schizophrenia

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Background: We use several complementary strategies to approach the pathobiology and genetics of schizophrenia including genetic association and family studies as well as animal and cell culture models.

Methods: We first aim to identify further schizophrenia genes in a large case-control and family-based study. 500 patients with schizophrenia according to DSM-IV (SCID) and 200 first degree relatives were included. Furthermore, 1300 community-based healthy volunteers without relevant somatic, and with no history of psychiatric disorders in themselves and in first-degree relatives entered the study. All subjects are screened by SCID and characterized by other specific instruments. High-throughput genotyping of candidate genes and linkage regions are under way and first results will be presented.
Results: Furthermore, we use endophenotypes as a complementary approach. The rationale for their use in gene discovery is that the endophenotypes associated with a psychiatric disorder are more elementary compared to clinical phenotypes. This also implies that the number of genes required to produce variations in these traits may be fewer than those involved in producing a psychiatric diagnostic entity. Our ongoing endophenotype project includes a broad range of schizophrenia endophenotypes. These comprise, among others, electrophysiological (e.g. P50 suppression and eye movement), neuropsychological (e.g. working memory, attention/vigilance, verbal learning and memory, visual learning and memory, speed of processing, reasoning and problem solving) and neuroimaging (fMRI during a working memory task) endophenotypes. We performed cognitive assessments (full scale IQ measurements) in over 1300 healthy subjects, schizophrenic patients and their first degree relatives. Furthermore, we assessed the other above-mentioned endophenotypes in over 300 subjects in this ongoing study. We present here data on the influence of common genetic variations on individual differences in cognitive abilities and discuss the findings in the context of schizophrenia and cognitive performance.
Discussion: Furthermore, we use an NMDA receptor antagonist animal model which mimics several aspects of psychosis to identify further candidate genes which can be used in our human studies. In our model, chronic, low-dose treatment with MK801 alters the expression of NMDA receptor subunits in a pattern similar to schizophrenia on the molecular level. On a cellular level, the number of parvalbumin- but not calretinin-positive interneurons was selectively decreased, a finding which parallels observations in post mortem brain from schizophrenic patients. On a functional level, recurrent inhibition of pyramidal cells was altered, as postulated from the histological findings. Finally, on a behavioral level, these animals showed cognitive deficits like disturbed working memory, which again parallels findings in schizophrenia. We used a functional genomic approach for the identification of hippocampal candidate genes for psychosis-related traits and identified several differentially expressed genes and pathways. These are under investigation in ongoing human genetic analyses.

163. Serotonin Transporter Polymorphism Influences the Prolactin Response to Tryptophan and Stress

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Background: Normal genetic variation in the genes that regulate serotonergic neurotransmission may be important determinants of normal and pathologic behavior. The insertion/deletion polymorphism in the serotonin transporter gene (5HTTLPR) is one of the most studied genetic polymorphisms which influence serotonergic function. The L (long) allele exhibits higher and the S (short) allele lower expression in in vitro model systems. Its involvement in serotonin-related behavioral disorders including depression, aggression, and other conditions is under active investigation. These studies suggest that 5HTTLPR influences on behavior interact in a complex way with ethnicity, gender and environment. The purpose of the present study was to determine if 5HTTLPR influences serotonergic function either as a main effect or moderated by gender, ethnicity or socioeconomic status (SES). We report the influence of 5HTTLPR on prolactin and cortisol reactivity to tryptophan infusion and stress challenges that were part of a larger study to evaluate the role of central serotonergic function in cardiovascular disease risk.
Methods: Subjects were 153 healthy volunteers stratified for race, gender and socioeconomic status (SES). They were admitted to the General Clinical Research Center at Duke for a 3 day protocol and were randomized to a serotonin enhancement or serotonin depletion limb of the study. On day 1, DNA was collected to determine 5HTTLPR genotype. One day 2 they received a saline infusion or tryptophan free drink, followed 3 hours later by a series of stressors alternated with rest periods. On day 3 they received a tryptophan infusion or tryptophan-free drink and the experiment was repeated. Blood was collected before and infusion, and after each stress and recovery period. Prolactin and cortisol were determined by RIA
Results: Tryptophan infusion (day 1 vs day 2, $n = 69$) increased PRL in a genotype-dependent manner ($p < .016$ for gene). Ss with L/L genotype ($n = 36$) showed the smallest increases, L/S ($n = 28$) were intermediate and S/S ($n = 5$) were the largest. There was a borderline gene x SES interaction ($p < .06$). Although the N was small, high SES S/S Ss tended toward higher PRL after tryptophan while low SES, S/S Ss had the lowest PRL. 5 HTTLPR similarly moderated the PRL responses to stress in the whole sample ($n = 153$) on day 2. Stress increased PRL modestly and 5 HTTLPR genotype influenced the response ($p < .03$ for gene). L/L Ss ($n = 71$) had the smallest and SS Ss ($n = 22$) had the largest response to stress. An interaction with SES occurred ($p < .038$ for gene X SES) that was opposite that seen after tryptophan: low SES Ss with S/S genotype ($n = 6$) had a larger PRL response to stress. 5HTTLPR genotype did not affect the cortisol response to either stimulus.
Discussion: These findings suggest that 5HTTLPR genotype predicts the magnitude of a neuroendocrine response to a serotonergic stimulus. The greater PRL response in S/S subjects might reflect a slower rate of clearance due to lower serotonin transporter expression. This could occur either in the hypothalamus or more rostral synapses involved in perception of stress and/or mediation of neuroendocrine function. This study also suggests that the interaction of SES with genotype depends upon the provocative test. In those with the ss genotype, high SES subjects showed exaggerated responses to a neutral or positive valence stimulus that enhances serotonin neurotransmission, while low SES subjects exhibited exaggerated responses to an aversive stimulus.

164. 5-HT_6 Receptor Stimulation Induces Neuroprotective Properties In Vitro and In Vivo

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Background: Recently, we have reported the identification of selective 5-HT_6 agonists which have furthered our understanding of the consequences of receptor stimulation and accordingly the role of the 5-HT_6 receptor in brain function. The 5-HT_6 agonist, WAY-208466, can increase extracellular GABA concentrations and effectively reduce glutamate release induced either by 50 mM KCl or sodium azide. Taken together, this neurochemical mechanism prompted the hypothesis that stimulating the 5-HT_6 receptor may have neuroprotective properties. Herein we report that 5-HT_6 receptor stimulation induces neuroprotective properties and neuroplasticity in vitro and in vivo.
Methods: Studies were performed using WAY-208466 (previously known as WAY-466) or WAY-181187 which are selective and potent 5-HT_6 agonists. In vitro paradigms of ischemic injury used to assess neuroprotective properties included oxygen glucose deprivation (OGD) in cerebellar granular cells (CGNs), potassium withdrawal in CGNs and acute anoxic deprivation in hippocampal slices. Neuronal plasticity was evaluated by measuring neurite outgrowth and survival in primary cortical and hippocampal cultures. In vivo neuroprotective properties were assessed using a transient middle cerebral artery occlusion model (tMCAO).

Results: Pretreatment of cultured CGNs with WAY-181187 or WAY-208466 followed by their exposure to OGD and a 24 hour recovery period resulted in a concentration-dependent reduction of neuronal cell death. WAY-181187 was effective from 0.01 to 1 μM with a maximal effect reducing neuronal cell death by 50%. The presence of WAY-181187 in CGNs during low potassium treatment significantly and in a concentration-dependent manner attenuated the amount of fragmented DNA used as a measure of apoptosis. The lowest concentration of WAY-181187 examined (0.3 μM) reduced cell death by 50% while almost total protection from apoptosis was found in the presence of 3 μM WAY-181187. WAY-181187 applied before and during acute anoxic deprivation in a rat hippocampal slice increased cell viability by 24% and 37%, respectively at 10 and 100 μM. Interestingly, WAY-208466 induced a significant increase in survival of primary cortical neurons in a concentration-related manner (0.01-10 μM) and induced a significant increase in neurite outgrowth compared to vehicle-treated controls. Notably, the effects on survival and neurite outgrowth were completely blocked by an equimolar concentration of the 5-HT_6 antagonists, SB-271046 or WAY-213155. In vivo studies performed using tMCAO revealed that WAY-181187 provided significant neuroprotective efficacy when administered post-induction of the ischemic insult. Neuroprotection was observed as a statistically significant reduction in the volume of infarcted brain tissue (27% and 30%, respectively at 10 and 15 mg/kg; iv).
Discussion: Notably over the last decade it has become apparent that there are neurodegenerative aspects of depression where it has been observed through imaging technology that there are volume reductions in both cortical and hippocampal tissue. Sheline and colleagues (2003) have noted that the correlation between reductions in hippocampal volume and duration of untreated depression is not observed following antidepressant treatment. It is interesting to speculate that $5HT_6$ receptor stimulation can account for neurite outgrowth and survival of neurons in the cortex and hippocampus following SSRI or SNRI drug treatments. Furthermore, the ability of a 5-HT_6 agonist to block or reverse ischemic injury suggests potential therapeutic application in the area of stroke.

165. Onset of Antidepressant Action and Acute Efficacy and Safety of Duloxetine Versus Escitalopram and Placebo in the Treatment of Major Depressive Disorder

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Background: Serotonin and norepinephrine uptake inhibitor antidepressants have been postulated to have higher remission rates and faster onset of action when compared with antidepressants that inhibit only serotonin uptake. We present results of a study comparing onset of efficacy (OE) and acute therapy phase efficacy and safety outcomes of the dual action antidepressant duloxetine with the serotonin-specific uptake inhibitor escitalopram.
Methods: Adult patients (n=684) with major depressive disorder were randomized to duloxetine 60 mg once daily (QD; N=273), escitalopram 10 mg QD (N=274), or placebo QD (N=137) for 8 weeks. The primary hypothesis was that the percentage of duloxetine-treated patients achieving OE at Week 2 was not inferior to (at least as great as) escitalopram. OE was defined a priori as at least a 20% decrease from baseline in the Maier subscale of the 17-item Hamilton Rating Scale for Depression (HAMD17) that was maintained at each visit. Secondary measures included: Hamilton Anxiety Rating Scale (HAMA), Clinical Global Impression of Severity (CGI-S) and the self-report Patient Global Impression of Improvement (PGI-I) scale. Standard safety measures were collected.
Results: The probability of meeting the OE criteria at Week 2 for duloxetine- and escitalopram-treated patients was estimated as 42.6% vs. 35.2%, respectively (p=.097). Placebo-treated patients met onset criteria (21.5%) at a significantly lower rate than duloxetine (p<.001) or escitalopram (p=.008). Secondary analyses found that probabilities of response for placebo, duloxetine and escialopram were 36.9%, 48.7%, and 45.3%, and for remission 27.7%, 40.1%, and 33.0%, respectively, with no statistically significant differences between groups. Mean change in total HAMD17 scores were -5.97 (0.58), -7.61 (0.42), and -7.22 (0.40), respectively with only duloxetine significantly different from placebo (p=.021). The two antidepressants had statistically significant changes in CGI-S and PGI-I scales when compared with placebo. The rate of discontinuation due to adverse events was similar for each group (placebo 5.8%, duloxetine 7.3%, escitalopram 5.1% ; p=ns). Treatment-emergent adverse events occurring significantly more frequently among duloxetine-treated patients when compared with those receiving escitalopram were nausea, dry mouth, vomiting, yawning, and irritability.
Discussion: In this study, duloxetine 60 mg QD had onset of sustained clinically meaningful improvement that was at least as fast as escitalopram 10 mg QD. The proportion of remitters and responders treated with placebo, duloxetine, and escitalopram was not significantly different at 8 weeks. However, duloxetine showed significant differences from placebo on the HAMD17 while escitalopram did not. Most other measures found similar changes with duloxetine and escitalopram.