Wednesday, December 14, 2005
Poster Session III - Wednesday

1. Design, Synthesis and Application of Novel Tropane-Based Photoaffinity Labels Toward the Identification of Binding Domains on the Dopamine Transporter

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Sponsor: Jonathan Javitch

Background: Irreversible ligands, derived from tropane-based dopamine uptake inhibitors, have been designed to covalently bind to discrete points of attachment on the dopamine transporter (DAT), via a photoactivated azido group. We previously have demonstrated that depending on the position of the azido substitution on the tropane ring system, covalent attachment can occur in different transmembrane regions suggesting that 1) the position of the azido attachment on the tropane ring dictates transmembrane domain labeling and/or 2) not all tropane-based DAT inhibitors bind to the same recognition site(s) on the DAT. Based on these early studies, we hypothesized that appending azido groups at numerous positions on different tropane-based DAT inhibitors (3-phenyl tropanes and benztropines) would provide the opportunity to identify points of attachment on the DAT, elucidate 3D-arrangement of the transmembrane domains, and direct future drug design. Herein, we report our progress in these pursuits.
Methods: All photoaffinity labels were designed to have azido groups attached to the N-, 2- or 3-position of the tropane ring of either the benztropine or 3-phenyltropane parent molecule. Synthetic strategies were undertaken based on modifications of previously published work (Agoston et al., 1997, Zou et al., 2001, 2003, Lever et al., 2005). Radiolabel displacement studies using [3H]WIN 35,428 in rat caudate putamen tissue were used to assess IC50 values of all final products and their immediate precursors.
Results: We initially prepared an N-substituted benztropine-based photoaffinity label [125I]GA 2-34, which covalently attached to the 1-2 transmembrane spanning region of the DAT. This was compared and found to be in contrast to the 4-7 transmembrane spanning region labeled by a 2-substituted-3-phenyltropane-based photoaffinity label, [125I]RTI 82 (Vaughan et al. 1999). Based on this early study we then synthesized a derivative of RTI 82 that had the same N-substituent as GA 2-34 (Zou et al. 2001). The resulting [125I]MFZ 2-24 (Lever et al 2005) has now been discovered to covalently link to a discrete domain in transmembrane domain 1-2 of the DAT (Vaughan et al 2005). In an attempt to provide a 2-substituted benztropine photoaffinity label, analogous to RTI 82, MFZ 4-40, (Zou, et al. 2003) was prepared as well as an additional compound in which the chain length at the 2-position was extended by two atoms. The resulting ligand, MFZ 6-107, showed lower DAT affinity (IC50= 131 nM) as compared to MFZ 4-40 (IC50= 55.6 nM), suggesting that an extended 2-position substituent was not well tolerated. Thus, a third 2-substituted analogue was derived using the linker length of MFZ 4-40 and the ester linkage of MFZ 6-107, which provided an improved synthetic strategy. In addition, a 3-beta-(3-iodo-4-azido-biphenylene)-analogue of RTI 82 was recently synthesized. This latest addition to our arsenal of DAT photoaffinity labels, JHC 2-48 (IC50= 15.1 nM) demonstrated the highest DAT binding affinity of any photoligand prepared to date, as well as moderate affinity for SERT (IC50= 109) suggesting its potential utility in labeling SERT for analogous protein structure studies.
Discussion: Radioiodination of these remaining photoaffinity labels will provide the necessary tools for elucidating binding domains on DAT (and SERT) of the tropane-based dopamine uptake inhibitors using immunoprecipitation and proteolysis strategies.

2. White Matter Changes in Cocaine Dependence

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Cocaine Dependence (CD) is a serious problem that often is refractory to treatment. Evidence suggests that CD is associated with white matter (WM) deficits that may underlie changes in mood, cognition and behavior. We studied 46 patients with CD and 17 controls using MRI at 1.5T to better characterize WM abnormalities in CD and to test whether they were reversible. Diffusion tensor imaging (DTI) was used for measurements in WM. DTI is an MRI method that provides measures (fractional anisotropy, FA) that are sensitive to WM pathology but do not provide insight into underlying mechanisms, as well as measures, such as axial (Dax) and radial (Dra) diffusivity, which can reflect specific microstructural changes.
Methods: Subjects were patients in a therapeutic community that closely monitored ongoing abstinence with random toxicology testing. Patients were subgrouped by duration of abstinence- <6 weeks (COC-S, n=15), 2-10 months (COC-M, n=15), > 1 year (COC-L, n=16)- and matched with non-using controls (CON). 8-direction DTI was acquired with a double spin echo, pulsed-gradient echo-planar imaging method that minimized distortion due to eddy currents. DTI maps were analyzed using both voxelwise methods and region of interest (ROI) approaches in separate analyses. Thresholding in voxelwise tests used a two-stage seed-cluster method that was designed to minimize false positive detections. Briefly abstinent subjects (n=7) also were scanned longitudinally to test whether cross-sectional differences were supported by changes in individuals.
Results: Subjects were well-matched on demographic variables. Number of years of cocaine use was similar in user subgroups and averaged ~10 years. When the whole CD cohort was compared with CON in voxelwise t-tests, patients showed mainly lower FA in most regions in which differences were found. In contrast, COC-S subjects showed more WM voxels with higher than lower FA, though these were concentrated in the inferior frontal WM. Whereas all patients showed significantly higher FA in frontal WM regions bilaterally, COC-S subjects also showed higher FA in ventromedial prefrontal WM regions that was not present in COC-L. COC-S subjects also showed higher FA in WM of the internal capsule adjacent to the right caudate nucleus that were not present in COC-L or when all CD patients were compared with CON. All patients showed lower FA in the substantia nigra and globus pallidus that was right-lateralized and lower FA in the deep WM of the temporal lobe that was left-lateralized. Moreover, frontal WM FA correlated positively with years of cocaine use and correlated negatively with duration of abstinence across all subjects. Comparison of COC-S and COC-L subjects showed predominantly higher frontal lobe FA in COC-S. Four regions with significant changes in FA in COC-S subjects, compared with COC-L, were also found in comparisons of FA in COC-S vs. CON, suggesting that these regions showed reversible deficits in CD. Longitudinal testing confirmed that increased FA in COC-S in frontal WM regions was reversible, but suggested that changes were more widespread. Analysis of tensor components showed that reversible changes were due to increased Dra over time. ROIs placed in frontal WM confirmed both the cross-sectional and longitudinal findings in frontal WM.
Discussion: This study showed reversible as well as persistent increases in frontal WM FA which must be viewed as deficits given the impaired functional status of patients tested. Elucidation of the basis of these changes may provide insight into the anatomical mechanisms in CD that may inform treatment.

3. Prediction of Alcohol Problems Using A Prospective Longitudinal Design Including Genotype

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Background: 1022 subjects from 448 families in the Collaborative Study of the Genetics of Alcoholism (COGA) were assessed at two time points separated by an interval of 4-5 years. The age of subjects at Time 2 was 17.4 + 3.4 years. At Time 2, 230 subjects were diagnosed with DSM IV alcohol dependence or abuse by personal interview and/or interview of a parent about the subject.
Methods: Characteristics at Time 1 were used to predict affected status at Time 2. Multilevel regression modeling was employed.
Results: Controlling for age, sex, and Time 1 diagnosis, parental affected status predicted Dependence/Abuse at Time 2 (Odds Ratio 1.4, Confidence Interval 1.0-1.8, F = 3.9, p<.05); the diagnosis of Conduct Disorder at Time 1 also predicted Dependence/Abuse at Time 2 (OR 1.7, CI 1.0-2.9, F = 4.4, p = .04). The diagnoses of Major Depression and Drug Dependence were also predictive. Affected Status at Time 2 was associated with Age at First Drink as reported at Time 1 (chi-square = 30.8, p < .0001). In younger adolescents, GABRA2 (the alpha 2 subunit of the GABA-A receptor) genotype may be expressed as conduct problems (F = 4.0, p = .02) and sometimes alcohol abuse. In older adolescents, GABRA2 genotype is associated with alcohol dependence (F = 11.2, p < .0003), especially when combined with Alcohol Dehydrogenase 4 genotype. CHRM2 haplotype (muscarinic cholinergic receptor 2) also predicts DSM IV alcohol dependence (F=6.12, p<.0056) along with separation anxiety and ADHD; this may be a separate vulnerability pathway.
Discussion: Additional subjects and additional vulnerability genes are now being studied prospectively. The intention is to develop models for risk and protective factors for the development of alcohol problems, incorporating genotypic information.

4. Casein Kinase 1 Epsilon Modulates Sensitivity to Stimulant Drugs in Both Mice and Humans: A Translational Genetic Study

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Sponsor: Harriet de Wit

Background: Liability to drug abuse may be partially determined by genetic differences in the sensitivity to the positive effects of drugs.
Methods: In order to identify genes that regulate sensitivity to methamphetamine (MA) in mice, we selectively bred for high or low MA-induced locomotor activity. We then used these mice to map quantitative trait loci (QTL) for this phenotype and also measured gene expression in the nucleus accumbens of drug-naive male mice from both selected lines, using Affymetrix microarrays and quantitative PCR. Based on the findings of our preclinical study, we conducted a human study examining the effect of polymorphisms in CSNK1E on the subjective response of 100 healthy human subjects to d-amphetamine (AMPH; 0, 10 and 20 mg; double-blind, counterbalanced order).
Results: Preclinical results: Statistically significant expression differences between the selected mouse lines were identified for several genes, including Casein Kinase 1 Epsilon (Csnk1e), and dopamine- and cAMP-regulated phosphoprotein of 32 kDa (Darpp-32). Csnk1e is known to phosphorylate Darpp-32, which plays a key role in the response to amphetamine and related stimulants. We used WebQTL (www.nervenet.org) to identify an expression QTL (eQTL) for Csnk1e on chromosome 15 (LOD=3.8) that co-mapped with one of our QTLs for the MA sensitivity (LOD=4.5), a region that has previously been associated with sensitivity to the stimulant effects of cocaine. These data suggested the existence of an allele that influences MA sensitivity by altering expression of Csnk1e. Human resutls: The primary outcome measure, subjects' ratings of whether they felt a drug effect (Drug Effects Questionnaire; DEQ), revealed a significant effect (p = 0.01) of a non-coding SNP (rs135745) on the subjective response to AMPH. Subjects with one or two copies of the C allele of rs135745 were more sensitive to the 10 mg dose of AMPH (p = 0.001). Subjects with different genotypes at this SNP also differed on secondary outcomes, including the "euphoria" scale of the ARCI, at the 10 mg dose.
Discussion: These findings demonstrate a powerful translational approach to studying pharmacogenetics in mice and humans, and offer a road-map for future investigations of genetically determined differences in drug sensitivity in humans. Further studies are needed to extend these findings, by investigating the relationship between the CSNK1E polymorphisms and drug abuse liability.

5. Cerebellar Vermis Participation in Cocaine-Related Behaviors

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Background: The primate cerebellar vermis (lobules II-III (anterior vermis-AV) & VIII-IX (posterior-inferior vermis-PIV) contains axonal dopamine transporter immunoreactivity (DAT-IR) and may be involved in cocaine-related behaviors. We analyzed previously acquired BOLD fMRI data to determine whether cocaine-related cues activated AV and PIV in cocaine users. We also analyzed PET imaging data from healthy subjects to determine whether ^{11}C-altropane, a DAT-selective cocaine congener, accumulates in AV and PIV.
Methods: BOLD fMRI studies involved 10 crack cocaine abusers (6 men, mean (SD) age=36±7 years) who abused cocaine at least biweekly, and 8 controls (3 men, 31±6 years old). BOLD regions of interest (ROI) were positioned by a trained rater blind to subject status (cocaine or control). PET imaging studies involved 11 adults (2 women, aged 23.9±0.9 years old). The initial 11 volumes acquired immediately after an ^{11}C-altropane bolus were summed to create blood flow images on which ROIs were manually traced by a trained rater.
Results: We found group differences in vermis activation ($F_{1,16}$=5.0, *P*=0.04) and cocaine subjects exhibited higher mean increases than controls (0.48% difference). BOLD activation magnitudes differed by vermis region ($F_{2,32}$=7.5, *P*=0.002); post-hoc Scheffe tests indicated greater activations in AV and PIV than in the DAT-IR-devoid posterior-superior vermis (PSV, *P*<0.01). At early time points following administration, time-activity curves indicated greater peak ^{11}C-altropane accumulation in PIV than in all other areas but putamen. There was a regional difference in PSV-normalized ligand accumulation ($F_{6,76}$= 12.2, *P*<0.001, excluding putamen). Post-hoc testing with Bonferroni correction for multiple comparisons indicated higher ^{11}C-altropane accumulation in PIV than in substantia nigra, frontal cortex, and all other cerebellar regions. Thalamic ^{11}C-altropane accumulation was equivalent to that in PIV, consistent with reports indicating DAT-IR enrichment in primate thalamus. We were unable to detect appreciable PIV accumulation using binding potential analysis, though that method may be less sensitive for detecting transient ligand accumulations in regions with low specific binding densities.
Discussion: Cocaine-related cues selectively activated the DAT-IR-enriched AV and PIV in cocaine users and a DAT-selective ligand accumulated in PIV in healthy subjects. Since PET data from cerebral blood flow (CBF) studies of healthy adults indicate higher cerebellar hemispheric than vermis CBF and homogenous intravermis CBF, while ^{11}C-altropane accumulation was lowest in cerebellar hemispheres and heterogeneous within vermis, it is unlikely that the excess PIV ^{11}C-altropane accumulation is due to CBF differences. While that

accumulation may represent labeling of other sites, a parsimonious conclusion is that DAT may be present in the DAT-IR-enriched PIV and that it may be a proximate site of action of cocaine and other drugs that interact with the DAT. Although we cannot definitively identify substrates mediating the effects noted presently, these data suggest that primate vermis areas enriched in DAT-IR may mediate some of cocaine's persisting and acute effects. Our findings warrant further studies to characterize cerebellar and vermis contributions to cocaine-mediated behaviors and to identify whether specific vermis substrates are targeted by cocaine and other stimulants. Support: DA16222, DA17324, DA14674, DA09448, DA14178, DA15116, DA14013, DA11558, DA15305, DA06303, RR00168.

6. Dysregulation of the Hypothalamic-Pituitary-Adrenal Axis in Alcohol-Dependent Self-Administering Rats

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Sponsor: George Koob

Background: Alcohol is a potent modulator of the stress system, and dysregulation of the stress axis may contribute to the development of alcohol abuse and alcoholism. Experimenter-controlled injection of alcohol has been shown to stimulate the hypothalamic-pituitary-adrenal (HPA) axis in rats, and the HPA response is blunted in animals previously exposed to chronic alcohol.
Methods: To test the hypothesis that impaired HPA function is associated with alcohol dependence; an animal model in which rats trained to self-administer ethanol was used. Wistar rats exhibited enhanced intake following chronic exposure to alcohol vapor ("dependent" rats) compared to trained rats not exposed to alcohol vapor ("non-dependent" rats). Serial blood samples were obtained via indwelling jugular catheters just prior to, during, and following oral self-administration of alcohol for measurement of plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone.
Results: Under basal conditions, dependent animals had lower ACTH and corticosterone compared to non-dependent animals. During self-administration, non-dependent animals displayed elevated ACTH and corticosterone levels in response to alcohol intake. Dependent animals had attenuated ACTH and corticosterone responses to alcohol intake, indicating that alcohol dependence is associated with dysregulation of the HPA axis.
Discussion: These data suggest that a dampened neuroendocrine state in dependent animals may lead to excessive drinking in order to establish normal endocrine function. Whether functional changes in the corticotropin-releasing factor system underlie the neuroendocrine tolerance observed in these animals is currently under investigation.

7. Sensitization-Prone C57BL/6J Mice Exhibit Reduced Dopamine D3 Receptor-Mediated Inhibition Relative to Sensitization-Resistant DBA/2J Mice

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Sponsor: Lei Yu

Background: Behavioral sensitization to psychostimulant drugs refers to the progressive and enduring increase in behavioral response following repeated drug administration, and may be associated with increased synaptic efficacy in mesolimbic dopamine pathways. Identification of genetic and molecular determinants of sensitization vulnerability may provide important insight into the molecular mechanisms that confer susceptibility to psychosis and substance dependence. Vulnerability to sensitization may be determined in part by inherited genetic factors, and the present study replicated previous findings that inbred C57BL/6 mice exhibit significantly greater locomotor sensitization following repeated treatment with amphetamine (AMPH) than inbred DBA/2J mice. This inbred strain difference therefore represents a useful model to elucidate genetic and molecular determinants of sensitization vulnerability. Because the D3 dopamine receptor (D3R) exerts an inhibitory effect on rodent locomotor activity, we hypothesized that drug-naive C57 mice would exhibit reduced D3R-mediated inhibition relative to drug-naive DBA mice.
Methods: D3R binding and mRNA expression were determined in the ventral stratum of C57 and DBA mice. Additionally, we examined the effects of specific D3R agonists and antagonists on novelty- and AMPH-induced locomotor activity in these two mouse strains.
Results: In support of our hypothesis, D3R agonist binding [3H]PD128907 was significantly reduced in the ventral striatum of naive C57 mice relative to naive DBA mice. D3R mRNA expression in the ventral striatum was similar in naive C57 and DBA mice, suggesting that reductions in D3R binding are mediated by post-transcriptional events. C57 mice also exhibited significantly elevated novelty-induced locomotor activity relative to DBA mice, and DBA mice exhibited a greater reduction in novelty-induced locomotor activity in response to the selective D3R agonist PD128907 (10 ug/kg) than C57 mice. Moreover, the selective D3R antagonist NGB 2904 (0.01, 0.1, 1.0 mg/kg) produced a dose-dependent increase in novelty-induced locomotor activity in DBA mice but not in C57 mice. Furthermore, NGB 2904 (0.01, 0.1, 1.0 mg/kg) produced a dose-dependent increase in locomotor activity induced by acute AMPH (1 mg/kg) in DBA mice, but not in C57 mice.
Discussion: Collectively, these data indicate that C57 mice exhibit significant reductions in dopamine D3R-mediated inhibitory function relative to DBA mice. Because down-regulation of D3R-mediated inhibition is known to occur in behavioral sensitization, differences in the functional reserve of D3R-mediated inhibition available to oppose locomotor stimulation could account for differing vulnerabilities to behavioral sensitization to dopamine-releasing drugs. The present findings take on additional significance in light of evidence that C57 mice more readily self-administer drugs that increase mesoaccumbens dopamine activity, including alcohol, morphine, nicotine, cocaine, and AMPH, relative to DBA mice.

8. Rats Maintain Ethanol Self-Administration into the Posterior Ventral Tegmental Area (VTA) Despite the Aversive Effects of Bicuculline

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Sponsor: Ting-Kai Li

Background: Ethanol (EtOH) is known to affect the GABA-A receptor system. Past research has indicated that the posterior VTA is a site that mediates the reinforcing properties of EtOH. The objective of this study was to examine the involvement of local GABA-A receptors in the reinforcing effects of EtOH within the posterior VTA of Wistar rats.
Methods: After surgery, Wistar rats were placed in standard two-lever (active and inactive) operant chambers and attached to the intracranial micro-infusion system. Rats were assigned to one of five groups (n = 5-7/group). Separate groups self-infused (FR1 schedule) artificial CSF (aCSF) or 50 uM bicuculline (BIC) for seven consecutive 4-hr sessions. Additional groups self-infused 200 mg% EtOH for the initial 4 sessions, co-infused 200 mg% EtOH plus 25 or 50 uM BIC during sessions 5 and 6, and 200 mg% EtOH alone during session 7. The last group of rats self-administered EtOH for the initial 4 session, 50 uM BIC alone during session 5 and 6, and EtOH alone during session 7.
Results: Rats readily self-administered EtOH into the posterior VTA, discriminated between active and inactive levers, and showed a place preference for the side of the operant chamber associated with the ac-

tive lever. In contrast, rats avoided self-administering BIC (fewer self-infusions than aCSF; 4.5 +/- 1 vs 11.1 +/- 1.7) and the side of the operant chamber associated with the active lever. Co-infusion of EtOH with BIC in sessions 5 and 6 did not alter total responding per session on the active or inactive lever, but did significantly alter the pattern of responding on the active lever. A history of EtOH self-administration did not alter the aversive properties of BIC in posterior VTA, because rats initially self-administering EtOH reduced self-administration behaviors and displayed a condition place aversion when BIC was administered alone.
Discussion: The results indicate that the reinforcing properties of EtOH in the posterior VTA (1) is not mediated by GABA-A receptors, (2) can maintain response-contingent behaviors even if those behaviors concurrently produce negative consequences.

9. Exposure to Cues Associated with Palatable Food Increases Immediate-Early Gene (IEG) mRNA and Proenkephalin (PENK) premRNA Expression in the Rat Brain
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Background: Cues associated with the intake of addictive drugs can precipitate drug craving and regional brain activation in human addicts as well as reinstatement of drug-seeking behavior and activity-dependent gene expression in the brain regions mediating this behavior in drug-experienced rats. The present study was undertaken in order to investigate whether contextual cues associated with the intake of a palatable food were capable of eliciting conditioned behavior and associated brain gene expression changes in a manner similar to cues associated with the administration of addictive drugs. In addition, we assessed the responsiveness of the endogenous, striatal opioid system to food cues since opioids have been shown to be involved in cue-elicited drug-seeking and food-intake behaviors.
Methods: We conditioned 16, *ad libitum* fed Sprague-Dawley rats to receive daily access to chocolate Ensure® in one of two environments distinct in visual, olfactory, and somatosensory cues over a 15-day period. In context A, the rats in the Ensure® cues group received access to Ensure® while the rats in the water cues group received access to water. In context B, the rats in the Ensure® cues group received access to water while the rats in the water cues group received access to Ensure®. Three days after the conditioning phase, rats were reintroduced into context A where half of the rats had received Ensure® and the other half of the rats had received water. All rats had been acutely fasted for 12 h before being tested. On the test day, rats had no access to either Ensure® or water and motor activity was measured by a photobeam activity system. After 45 min in context A, rats were anesthetized and sacrificed. The expression of the IEGs Arc, Homer1a, zif268, NGFI-B, and c-Fos was assessed in a variety of brain regions via *in situ* hybridization. The expression of the PENK gene was also assessed via *in situ* hybridization but with a probe directed against intron 1 of the PENK premRNA in order to more sensitively measure its transcription.
Results: The rate of and total Ensure® intake and body weight gain were no different between the two groups during training. On the test day, the Ensure® cues group exhibited significantly higher motor activity compared to the water cues group. This conditioned motor activation was associated with increased expression of all of the IEGs in prefrontal cortical subregions, cingulate cortex (except zif268), hippocampal subregions, the core and shell of the nucleus accumbens, striatal subregions, and the basolateral but not the central nucleus of the amygdala. Ensure® cues increased PENK premRNA levels only in ventral striatal subregions.
Discussion: Since many of these IEGs have been implicated in learning and memory, the expression pattern observed here suggests that systems involved in executive function, spatial and episodic memory, and the assessment of emotional valence and motivational value are not only engaged by food cues but, upon non-reinforced presentation of such conditioned stimuli, adapt in order to store information about the reliability of these cues in predicting the availability of food. Our results also show that the striatal opioid system, which mediates some hedonic and motivational properties of both food and addictive drugs, is responsive to food cues. Since similar behavior and gene expression patterns are observed in response to cues associated with food or addictive drugs, the above considerations may have important implications for the pharmacological and cognitive behavioral treatment of eating disorders, obesity, and addiction.

10. Ontogeny of the HPA-Axis and Monoamine Response to Acute Ethanol Challenge in Male and Female Adolescent Rhesus Macaques
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Sponsor: Susan Swedo

Background: In rodents, ethanol is known to activate the hypothalamic-adrenal-pituitary (HPA) axis, as well as to stimulate changes in monoamine levels in the brain, following acute administration. Essentially, acute administration of ethanol increases plasma ACTH and corticosterone levels, as well as increases norepinephrine and dopamine levels in specific brain regions. The effects of ethanol on serotonin levels in the brain are less clear. Studies in rodents have also demonstrated age and sex differences in ethanol's effects on the HPA axis and on the central nervous system. This study investigated age-dependent variation in the effects of acute ethanol administration on HPA-axis function and central monoamine turnover in male and female adolescent rhesus macaques.
Methods: Alcohol-naive male (N = 54) and female (N = 70) rhesus macaques, ranging in age from 20 to 48 months, were give a single intravenous dose of ethanol (2.2g/kg for males, 2.0g/kg for females). Plasma samples were collected at 5, 10, and 60 minutes post-injection. Cerebrospinal fluid (CSF) samples were collected one month prior to injection (baseline) and again at 60-minutes post-injection. Associations between age at time of injection and 1) peak plasma levels of ACTH and cortisol, 2) area under the curve (AUC) values for ACTH and cortisol, and 3) CSF concentrations of 5-HIAA, MHPG, and HVA, were analyzed using multiple regression techniques.
Results: Controlling for rearing condition (mother-reared vs. nursery reared), males showed a significant positive association between age and peak ACTH levels (partial $r = 0.569$, $p = 0.0005$), and a significant negative association between age and peak cortisol levels (partial $r = -0.440$, $p = 0.0092$). Similar results were found for AUC for both ACTH and cortisol in males. Females on the other hand exhibited no significant associations between age and either cortisol or ACTH. For the monoamine metabolites, males and females showed similar negative associations between age and percent change from baseline in HVA (males: partial $r = -0.463$, $p = 0.0008$; females: partial $r = -0.459$, $p < 0.0001$) and age and percent change from baseline in MHPG (males: partial $r = -0.536$, $p < 0.0001$; females: partial $r = -0.454$, $p < 0.0001$). Females also showed a significant negative association between age and percent change from baseline in 5-HIAA (partial $r = -0.356$, $p = 0.0029$).
Discussion: These results suggest that both age and sex are important factors when investigating the effects of ethanol on HPA axis activity and central nervous system functioning. Furthermore, since adolescence is such a crucial time period for the development of alcohol use and alcohol-related problems, it is important to understand the development of mechanisms that may underlie ethanol's effects on mood, behavior, and motor performance during this time. This research was carried out in accordance with the Declaration of Helsinki

and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

11. Aripiprazole Attenuates Cocaine-Seeking Behavior in an Animal Model of Relapse

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Background: Aripiprazole (Abilify) is an atypical antipsychotic drug characterized by partial agonist activity at dopamine (DA) D2 and D3 receptors and a low side effect liability profile. Based on the idea that its antipsychotic pharmacological properties involve the "stabilization" of mesocorticolimbic DA activity, we hypothesized that aripiprazole may attenuate relapse in abstinent cocaine users. To test this hypothesis, we assessed the effects of aripiprazole HCl on conditioned cue-induced and cocaine-primed reinstatement of cocaine seeking behavior in an animal model of relapse.
Methods: Male, Sprague-Dawley rats were trained to lever press during 2 hr daily sessions for intravenous cocaine (0.5 mg/kg/infusion) paired with the presentation of a compound stimulus cue (light + tone) for 12-14 days. Responding was then allowed to extinguish in the absence of either cocaine or the drug-paired cue. Prior to reinstatement of cocaine seeking behavior (i.e. responding on the previously cocaine-paired lever) either in the presence of the compound stimulus or after a cocaine priming injection (10 mg/kg, IP), rats received an injection of aripiprazole HCl (0.25, 0.5, 1.0, 2.5, 5.0, and 15 mg/kg, IP) or vehicle.
Results: Vehicle-treated animals showed robust reinstatement of lever pressing responses on the previously reinforced lever (cue presentation increased responding 4-5x over extinction levels, while cocaine priming increased responding 7-8x over extinction levels). Pretreatment with aripiprazole significantly attenuated both cue and cocaine-primed reinstatement in a dose-dependent manner. In addition, doses of aripiprazole that were effective at attenuating reinstatement failed to produce catalepsy or reduce spontaneous locomotor activity.
Discussion: These findings from an animal model of relapse support the contention that aripiprazole may be an effective and relatively safe therapeutic agent for the prevention of relapse in abstinent cocaine users, possibly by stabilizing dopamine agonist tone in D2/D3-rich brain areas like the nucleus accumbens. Furthermore, given its effective antipsychotic effects, aripiprazole may be particularly useful for individuals with comorbid psychosis, such as schizophrenia. These studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health. This research was supported by NIH grants RO1 DA10462 and P50 DA16511.

12. Chronic, but Not Acute, Desipramine Treatment Prevented Nicotine Withdrawal and Decreased Nicotine Self-Administration in Rats

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Sponsor: Athina Markou

Background: Change in noradrenergic function is one of the abnormalities hypothesized to be involved in depression. Nicotine withdrawal is characterized by depression-like symptomatology (anhedonia) that similarly to non-drug-induced depressions may also be partly mediated by dysregulations in noradrenergic transmission. Clinical studies investigating the effects of antidepressant norepinephrine reuptake inhibitor treatment in smoking cessation have provided promising results. Nicotine dependence is maintained by both the reinforcing effects of acute nicotine and the negative depression-like aspects of nicotine abstinence. The present study evaluated the effects of acute and chronic administration of desipramine (DMI), a relatively selective norepinephrine reuptake inhibitor and antidepressant, on both the reinforcing effects of nicotine and the depression-like aspects of nicotine withdrawal in rats.
Methods: A rate-independent current-intensity discrete-trial threshold procedure was used to assess brain self-stimulation reward thresholds in rats prepared with electrodes in the lateral hypothalamus. Nicotine dependence was induced by continuous nicotine infusion via subcutaneous osmotic minipumps (3.16 mg/kg/day, free base). Observational methods were used to assess somatic signs of nicotine withdrawal. DMI was administered acutely under baseline conditions and during nicotine/saline withdrawal induced by cessation of 7 day nicotine/saline exposure. In another study, chronic DMI (15 mg/kg/day, salt) administration through minipump began after 7 days of nicotine/saline exposure and was continued together with nicotine/saline delivery for 14 days. Then, the nicotine/saline minipumps were removed while DMI/vehicle minipumps remained in situ. Brain reward thresholds were assessed daily while somatic signs were counted 24 hours after pump removal. Other rats were prepared with intravenous catheters and allowed to self-administer nicotine (0.015 mg/kg/inf, free base). After the establishment of stable self-administration, these rats were prepared with minipumps containing DMI (15 mg/kg/day)/vehicle and allowed to self-administer nicotine for an additional 12 consecutive days.
Results: In nicotine-naïve rats under baseline conditions, DMI (0, 0.5, 1.0, 2.0 mg/kg) had no effect on brain reward function. Acute DMI (2 mg/kg) administration had no effect on brain reward thresholds of rats withdrawing from 7 days of exposure to nicotine/saline. Interestingly, chronic DMI treatment prevented the threshold elevations associated with nicotine withdrawal and partially attenuated the increased number of somatic signs of withdrawal. There was no effect of either acute or chronic DMI treatment on response latencies in saline- or nicotine-exposed rats after pump removal. Finally, chronic DMI treatment significantly decreased nicotine self-administration throughout the 12-day treatment period.
Discussion: Blockade of nicotine self-administration, prevention of withdrawal-associated threshold elevations and partial attenuation of the somatic signs of nicotine withdrawal with chronic, but not acute, DMI treatment suggest that noradrenergic neurotransmission is involved in mediating both the reinforcing effects of nicotine and the affective depression-like aspects of nicotine withdrawal. These data suggest that DMI or other norepinephrine reuptake inhibitors may be anti-smoking treatments that would target different aspects of nicotine dependence by reducing both the reinforcing effects of acute nicotine and alleviating the depression-like aspects of nicotine withdrawal in humans. Finally, these data suggest that there may be homology between drug- and non-drug-induced depressions.

13. Positron Emission Tomographic Study of Regional Brain Metabolic Responses to a Serotonergic Challenge in Major Depressive Disorder with and Without Comorbid Alcohol Dependence

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Sponsor: Thomas Cooper

Background: Major depression (MDD) and alcohol dependence (alcoholism) are often comorbid. Depressed subjects with alcoholism have more chronic morbidity and mortality than individuals with either diagnosis alone. The serotonergic system is involved in the pathophysiology of both depression and alcoholism. Studies have used fenfluramine challenge to elucidate regional serotonergic function in addition to measures of resting regional glucose metabolic rate responses (rCMRglu). Fenfluramine causes a release of serotonin, consequent changes in rCMRglu can be measured by 18FDG and positron emission tomography (PET). This is the first study con-

trasting these changes in responses to a serotonergic challenge in major depressive disorder with and without comorbid alcoholism.
Methods: Twenty-eight patients with MDD without a history of alcoholism and 15 patients with MDD and comorbid of alcoholism were enrolled in this study. All met DSM-IV criteria for a current major depressive episode in the context of major depressive disorder. Subjects received placebo on the first day and fenfluramine on the second in a single blind design. A bolus injection of approximately 5 mCi 18FDG was administered three hours after the administration of placebo/fenfluramine. A Siemens ECAT EXACT 47 scanner was used to acquire a 60-min emission scan in 2D mode as a series of twelve 5-min frames. Regions of significant differences in rCMRglu between depressed subjects with and without a history of alcoholism on placebo day and fenfluramine day were evaluated using Statistical Parametric Mapping.
Results: Patients with MDD without comorbid alcoholism had lower aggression and hostility scale scores compared to individuals with MDD and comorbid alcoholism. Prolactin levels rose significantly after fenfluramine administration compared to after placebo but there was no difference in prolactin responses to fenfluramine administration between the two groups. We found no difference in rCMRglu between the two groups after placebo or fenfluramine administration. Because our previous study suggested that there were differences in rCMRglu between MDD patients with and without comorbid borderline personality disorder (BPD), we were also interested in reanalyzing the data only for MDD subjects without BPD (MDD/no BPD). When we repeated the analysis excluding patients with BPD from both groups, patients without comorbid alcoholism still had lower aggression and hostility scale scores compared to their counterparts. When comparing rCMRglu after placebo administration in MDD/no BPD patients with and without comorbid alcoholism, we found an anterior medial prefrontal cortical area where MDD/no BPD patients with comorbid alcoholism had more severe hypofrontality than MDD/no BPD patients without alcoholism. This area encompassed the left medial frontal and left and right anterior cingulate gyri. This group difference disappeared after fenfluramine administration.
Discussion: Higher aggression and hostility in MDD patients with alcoholism may be related to greater hypofrontality in this patient group. The fact that the observed group difference disappeared after the fenfluramine challenge suggests that serotonergic mechanisms play a role in the observed differences between the groups. It also suggests that serotonergic antidepressants may be useful in the treatment of MDD patients with comorbid alcoholism.

14. NMDA Receptors Mediate Some of the Lingering Behavioral Deficits of Repeated Ethanol Exposure in Adolescent Rats

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Sponsor: Herbert Barry

Background: Earlier studies have shown that ethanol treatment differentially affects water maze performance in adolescent and adult rats. The present study was undertaken to examine the age-specific reversal of ethanol-induced deficit in water maze performance, and to determine the cellular mechanisms underlying the behavioral deficits. The hypothesis tested was that adolescent ethanol-induced cognitive dysfunction is associated with alterations in the regulation of N-methyl-D-aspartate (NMDA) class of glutamate receptors.
Methods: Adolescent male rats were subjected to repeated ethanol or saline treatment. Experimental rats were injected daily with 2 g/kg ethanol (intraperitoneally) for five consecutive days (Days 1-5) and tested in the reference memory task of the Morris water maze thirty minutes after ethanol treatment; control rats received isovolumetric saline. On the last training day, all rats were subjected to the probe trial and then tested in the visual cued task. After an ethanol-free period of 4-25 days, rats were retested in the water maze. Groups of experimental and control rats were sacrificed, brains/brain regions were frozen immediately. Synatsomal membranes were prepared from frozen brain regions and saturation radioligand binding assays were carried out using [3H]MK-801. NMDA receptor subunit (NR1, NR2A, NR2B) protein levels were measured immunohistochemically in ethanol-treated and saline-treated coronal brain sections.
Results: Compared to age-matched saline controls, ethanol-treated rats showed significantly higher latencies and swam greater distances to find the hidden platform. In the probe trial, compared to saline rats, ethanol rats spent less time in the target quadrant. But there was no significant difference in the swim speed or performance in the cued visual task between ethanol- and saline-treated rats. Ethanol-treated rats continued to do poorly on all retest days. Rats treated with ethanol showed upregulation of [3H]MK-801 labeled NMDA receptor. This was associated with increases in specific NMDA subunit protein levels.
Discussion: Adolescent rats exposed to repeated ethanol treatment showed significant deficits in water maze performance. Even after several weeks of post-ethanol exposure period, ethanol-treated rats failed to catch-up to control rats in their water maze performance. NMDA receptors were upregulated in the ethanol-treated rats. Together, these data suggest that the NMDA receptor plays a significant role in adolescent ethanol-induced lingering behavioral deficits.

15. Selective Enhancement of Limited Access Ethanol Intake in Isolate-Housed Alcohol-Preferring (P) Rats

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Sponsor: Cindy Ehlers

Background: Environmental factors, such as adverse early life experiences and peer interactions can contribute to the development of alcohol drinking patterns and use disorders. In animals, peer separation/isolation has been used as an environmental intervention that has been shown to influence drinking behaviors in rodents.
Methods: In the present study, rats selectively bred for alcohol preference (i.e., alcohol preferring P rats and non-alcohol preferring NP rats) were housed in pairs or singly from an early age (i.e., 30-40 days old). After 13 weeks of isolate or pair housing, ethanol intake was assessed over a 14 day period in a 24-hour home cage preference. The initiation and maintenance of ethanol drinking during was then assessed during 20 minute limited access sessions.
Results: There were no significant effects of housing condition on ethanol intake or ethanol preference scores during 24 hour access. Isolate-housed P rats drank significantly more than paired-housed P rats across a range of sucrose, sucrose-ethanol, and ethanol solutions [housing condition x solution interaction, $p < 0.05$] during limited access drinking sessions. Although isolate-housed NP rats tended to drink less than pair-housed NP rats, these differences did not reach statistical significance. Significant positive correlations were also reported when assessing 10% ethanol intake during limited access sessions in relation to motor activity assessed prior to the initiation of limited access drinking sessions.
Discussion: Taken together, these data demonstrate an important gene x environment interaction on limited access ethanol intake. Specifically, isolate-housing was found to selectively enhance ethanol drinking in rats with a genetic predisposition for high ethanol intake. These data suggest that environmental factors might be more likely to facilitate alcohol misuse/abuse in individuals with a positive family history of alcoholism.

16. Amphetamine Effects on Amygdala Responses to Affective Pictures in Healthy Volunteers

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Background: Amphetamine is a monoamine agonist with acute mood and behavioral effects, the neural bases of which are just beginning to be studied using fMRI in healthy volunteers. The present fMRI study was designed to determine whether d-amphetamine modulates responses to positive and negative affective pictures, in previously drug-naive individuals, in order to determine how d-amphetamine alters the processing of emotionally-relevant stimuli during first drug exposure.
Methods: Ten healthy male participants received d-amphetamine (20 mg oral) and placebo 90 minutes prior to fMRI scanning and presentation of the International Affective Picture Set task in a two-session, double-blind, within-subjects design. Participants were preselected based on personality traits that have been found to modulate the effects of the drug in other samples, as assessed by a standardized personality inventory with an orthogonal factor structure (Multidimensional Personality Questionnaire, Brief Form).
Results: Preliminary data (N=10) indicate that compared to placebo, d-amphetamine decreased the intensity of fMRI activation in the amygdala, and the magnitude of this amphetamine-induced decrease was greatest during the negative blocks of the task (drug x valence interaction; empirically derived left amygdalar cluster; $F(2,8) = 6.59$, $p < .05$). These findings differ from those previously obtained using negative faces (Hariri et al., 2002). These data suggest that amphetamine may differentially modulate processing of physical versus social negative stimuli by the amygdala.
Discussion: Initial data from this ongoing pharmacological fMRI study indicate that d-amphetamine results in a shift away from processing negative stimuli related to physical threat. Potential individual differences in the magnitude of this effect will be discussed. Viewed in tandem with previous findings from other laboratories, these data suggest that amphetamine has complex effects on the processing of negative emotional stimuli in healthy volunteers, with the potential for decreasing the processing of stimuli related to imminent physical harm. Implications for the transition from first to continued use of psychostimulants, and risk-taking behavior under drug, is discussed.
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17. The Effects of MDMA (Ecstasy) on Sleep Architecture and Mood in Humans

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Sponsor: Chris-Ellyn Johanson

Background: Anecdotal reports indicate that MDMA produces dysphoric alternations in mood for several days following use, suggesting a type of withdrawal effect. However, it is not clear whether these reports are accurate. Reasons for lack of accuracy could include the retrospective nature of the reporting, attribution to MDMA (despite not knowing what drug was consumed), or as a direct consequence of sleep disruption.
Methods: In the present study, recreational MDMA users, 18-25 yrs, without psychiatric disease or drug dependence and in good health participated in a three session experiment. On 3 sessions, with 3 nights (baseline, treatment, and recovery) and 2 days (after night 2 and 3) per session, participants on the treatment night (night 2) received placebo or 2 mg/kg MDMA at 1800 hr, or a restricted bedtime (0300-0700 hr). Bedtime was 8 hrs on non-restriction nights and standard nocturnal polysomnograms (NPSGs) and multiple sleep latency tests (MSLTs) to measure daytime sleepiness were collected. In addition mood effects (VAS, POMS, and ARCI) were measured following drug administration as well as for 48 hrs following drug or placebo administration.
Results: No baseline differences were seen on NPSG measures prior to treatment nights. Total sleep time was reduced with MDMA relative to placebo by 29% (5.2 vs 7.3 hrs, $p<.05$) due to increased sleep latency (112.3 vs 14.9 min, $p<.04$). Sleep time in the bedtime restriction was 50% (3.7 hrs) of placebo. REM was significantly decreased to 3.5% following MDMA compared to 19.2% after placebo and 19.3% after sleep restriction. MDMA did not alter other sleep stages. Mean daily sleep latency on the MSLT was reduced after 4 vs 8 hr bedtime by 54% (6.8 vs 14.8 min, $p<.02$); MDMA had no significant effect on mean daily sleep latency (14.3 vs 14.8 min). On the recovery night all measures returned to baseline. MDMA produced typical subjective effects one to four hours post-administration. Daily mood assessments over the next 48 hrs indicated no disruptions for MDMA compared to placebo but disruptions in mood were observed following sleep restriction.
Discussion: This study, the first prospective assessment of sleep following MDMA suggests dramatic decreases in REM sleep following MDMA. In addition, it does not appear that the dysphoric mood reported following MDMA is not a function of a single dose of MDMA.
Supported by DA14874 and Joe Young Sr funds from the State of Michigan.

18. Positive and Negative Transcription Regulation of NR2B Gene in Basal and Ethanol-Induced Gene Expression in Fetal Cortical Neurons

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Background: The N-methyl-D-aspartate (NMDA) receptor mediates the excitatory neurotransmission in the CNS, is involved in learning and memory formation, and in the refinement of synaptic connections during development. Previous studies in our laboratory and other groups have demonstrated that long-term ethanol treatment caused NR2B subunit transcription up-regulation. The development of physical dependence on ethanol involves up-regulation of NMDA receptors, especially their NR2B subunits. In present study we have investigated the role of transcription factors activating protein 1 (AP-1) and neuron-restrictive silencer factor (NRSF) in regulating NR2B gene in basal and following long-term ethanol treatment.
Methods: Primary cultured cortical neurons exposed to 75 mM ethanol for 5 days. Electrophoretic mobility shift assay, Chromatin Immunoprecipitation Analysis, luciferase reporter assay, site-directed mutagenesis, transfection, and DNA affinity precipitation assay were used in this study.
Results: There is a positive regulative area located between base pair -1107 and -1084 and a negative regulative element is between base pair -1407 and -2741. A putative AP-1 and five NRSE binding sites were identified by the specific binding of AP-1, CREB and NRSF protein in vitro and in vivo respectively. Luciferase reporter system and mutation analysis demonstrated the enhancing role of AP-1 and repressing role of NRSF on the regulation of NR2B transcription and mediation of ethanol effect.
Discussion: The results suggest that NR2B transcription regulation subjected to both positive and negative regulation mediated by AP-1 and NRSF. More importantly, in this study, we found that long-term ethanol treatment cause NR2B transcription up-regulation through both AP-1 and NRSF regulation by affecting the formation of complexes.

19. An Open Label Pilot Trial of Atomoxetine and Four-Session Motivational Interviewing for Cannabis Dependence

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Sponsor: Past Travel Awardee, PMRTP, 2004

Background: Cannabis is the most widely used illicit drug in the U.S. According to the 2000 U.S. National Household Survey on Drug

Abuse, cannabis dependence afflicts 1.6 million American adults and an additional 1.2 million adults meet criteria for cannabis abuse. In many respects, the changes in executive functioning exhibited in marijuana users resemble the deficits seen in patients with attention deficit hyperactivity disorder (ADHD). Thus far, behavioral treatment trials for marijuana dependence have not been able to show significant sustained efficacy. There are new classes of medications that can specifically target the endocannabinoid system, but their application to the treatment of marijuana dependence remains far off. One potential approach for the pharmacological treatment of cannabis dependence would be to exploit the similarities between the cognitive deficits from cannabis and those of ADHD. Atomoxetine is a presynaptic norepinephrine transporter inhibitor approved for the treatment of ADHD. If Atomoxetine could compensate for the ADHD-like cognitive impairments from cannabis use these patients may derive greater benefit from psychosocial and behavioral interventions.
Methods: N = 21 treatment seeking volunteers were recruited from the community to participate in a 12-week open-label ascending dose trial of atomoxetine (25 to 80mg) and brief 4-session MI for cannabis dependence. Patients were excluded for DSM-IV mood, anxiety, psychotic or other substance use disorders and all had a diagnosis of primary cannabis dependence. Subjects attended weekly medication and data collection visits. Subjects completed a battery of cognitive, mood and self-report measures and submitted weekly urine drug screens for quantitative and qualitative THC and quantitative Creatinine levels at each study visit.
Results: Out of 21 subjects recruited, 16 started medication and 9 subjects completed the 12-week trial (56% completion rate). Subjects had modest within trial self-reported reductions in marijuana use and problems related to marijuana use (detailed analysis pending). Although 50% of participants reported at least one week of total abstinence, only 19% had UDS (THC qualitative) confirmed abstinence. Overall attendance for weekly visits was 63%. Average marijuana use across the sample was $7.12 per day. 62.5% of subjects co-administered tobacco and marijuana. On preliminary examination, subjects reported few significant cognitive problems on self-report measures (CAARS), but did exhibit some impairment in executive functioning and measures of cue-induced conditioning on implicit tasks. The rate of gastrointestinal adverse events was high (62.5%) across the sample even at low doses.
Discussion: On preliminary analysis atomoxetine, in this group of urban cannabis dependent subjects, does not seem to have an effect on marijuana abstinence. There may be some improvement on specific cognitive domains, but it is not certain is it is medication related. A detailed analysis of the urine THC/Creatinine ratio may shed additional light on the self-reported reductions in marijuana use during the trial. The dose and frequency of brief motivational interviewing style interventions in pharmacological trials for cannabis dependence requires further investigation. The role of nicotine co-administration and the high incidence of gastrointestinal side effects in cannabis dependent subjects on atomoxetine merits further investigation.

20. Brain Stimulation Reward Thresholds Are Altered by Viral Vector-Mediated Elevations of GluR Subunits Within the Nucleus Accumbens Shell

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Background: Repeated cocaine exposure leads to increases in the AMPA-glutamate receptor subunit GluR1 in the nucleus accumbens (NAcc). Moreover, elevations of GluR1 within the shell of the NAc (NAs) decrease drug reward whereas elevations of GluR2 increase drug reward. The effects of alterations in these GluR subunits on other types of rewards, however, are less clear.
Methods: Here, we microinjected herpes simplex viral (HSV) vectors to selectively increase GluR1 or GluR2 expression in the NAs in order to determine if the rewarding impact of lateral hypothalamic brain stimulation was changed using intracranial self-stimulation (ICSS). Rats were implanted with lateral hypothalamic stimulating electrodes and bilateral guide cannula aimed at the NAs. Rats were then trained on a ″rate-frequency″ ICSS procedure in which the minimum amount of stimulation required to sustain the behavior (i.e., threshold) can be calculated. Once thresholds were stable for 5 days, the HSV-GluR1, HSV-GluR2 or HSV-LacZ (control) vectors were microinjected bilaterally into the NAs.
Results: Microinjections of HSV-GluR1 caused immediate but transient increases in thresholds two hours after gene transfer, reflecting a decrease in the rewarding effects of lateral hypothalamic brain stimulation. Thresholds returned to baseline levels on the subsequent two days, then increased on days 3-5, and gradually returned to baseline levels by day 8. Microinjections of HSV-GluR2 had no effect two hours after gene transfer, but they decreased thresholds at two days, reflecting an increase in the rewarding effects of lateral hypothalamic brain stimulation. Thresholds returned to baseline levels on day 4 and remained stable through day 8. Microinjections of HSV-LacZ had no effect on thresholds.
Discussion: These data suggest that elevated expression of GluR subunits in the NAs alters the rewarding properties of lateral hypothalamic brain stimulation. Considering that acute drug withdrawal also increases ICSS thresholds and elevated expression of GluR1 in the NAs makes low doses of cocaine aversive, our findings suggest that elevated expression of GluR1 in the NAs may be a neuroadaptation that contributes to experience-dependent reductions in reward or increases in aversion. In contrast, elevated expression of GluR2 in the NAs may contribute to experience-dependent increases in reward. These 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. DA018037 (to MT) DA12736 (to WC)

21. The Impact of Screening, Brief Intervention, and Referral to Treatment (SBIRT) on Emergency Department(ED) Patients' Alcohol Use

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Many ED patients are high risk or dependent drinkers. This study (translational research in a real life setting) examined the impact of screening, brief intervention and referral to treatment (SBIRT) on ED patients' drinking behavior at 3 months.
Methods: ED patients meeting NIAAA criteria for high risk drinking were recruited from 14 sites nationwide (control group-Spring, 2004; intervention group-Summer, 2004). All enrollees received a list of local referral resources; intervention group patients also participated in a 15 minute negotiated interview (see www.ed.bmc.org/SBIRT) and ED staff referred them directly for treatment if indicated. Patients completed 3-month follow-up surveys with a telephone Interactive Voice Response (IVR) system. SUDAAN was used to adjust for weighted sampling.
Results: 26% of screened ED patients met inclusion criteria. A total of 1137 patients were enrolled across14 sites (561 intervention, 576 control), with 62% male, 50% Black, and 38% White, and a mean age of 37. At baseline intervention-i and control-c groups were similar in demographic characteristics, number of drinks on one occasion (mean-i 5.00, mean-c 5.12) and maximum drinks (mean-i 7.51, mean-c 7.33). The follow-up rate was 62% (n=687). Male gender was the only significant predictor of attrition. At 3-month follow up, intervention group enrollees reported greater reductions than controls in the typical number of drinks per occasion (mean-i 4.06, mean-c 4.75) and the maximum number per occasion (mean-i 6.48, mean-c

7.15). Regression analyses adjusting for the clustered sampling design showed significant effects of the intervention over the past 30 days on the typical number of drinks per occasion (beta -.63; CI -1.06,-0.19; p<.01) and the maximum number of drinks consumed on any one occasion (beta -.68; CI -1.33,-0.02; p<.05).
Discussion: ED screening and brief intervention with referral for treatment may foster self-reported decreases in alcohol use among high risk drinkers.

22. Effects of Acute Plasma Tryptophan Depletion on Serotonin Receptor Binding Using PET in Healthy Controls: Implications for Alcoholic Brain Disease
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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: Alcohol dependence (AD) is an etiologically heterogeneous syndrome determined by a complex interaction of genetic and environmental factors. We propose to investigate monoamine dysfunction in alcoholic patients by using acute tryptophan (TRP) depletion (ATD) and PET imaging. Differences in 5-HT turnover (synthesis, metabolism, and release) in alcoholics may be involved in reinforcing responses to ethanol and alcohol seeking behaviors. To examine this question, we first studied 5-HT turnover and 5-HT1A receptor occupancy in healthy volunteers. We report the results of the first 5 subjects in this cohort. We hypothesize that diminished 5-HT release following ATD leads to decreased competition for 5-HT1A receptors by endogenous 5-HT, and greater 5-HT1A receptor binding of the radioligand [18F]FCWAY, a 5-HT1A receptor antagonist. ATD lowers brain 5-HT synthesis and release by decreasing plasma and brain TRP concentration. We administered a TRP-deficient amino acid mixture to acutely lower plasma TRP, the precursor for 5-HT synthesis. In humans, ATD decreases plasma TRP concentration, with a nadir occurring approximately 5 to 6 hr after drink administration. 5-hydroxyindoleacetic acid (5-HIAA), the principal metabolite of 5-HT and a neurochemical marker of neuronal 5-HT metabolism, also decreases.
Methods: [18F]FCWAY PET studies with concurrent CSF/plasma sampling were performed in 5 healthy volunteers, in a within-subject repeated measures design. The baseline PET scan was done before ATD on day 1. The active condition scan was done on day 2, 16 hr after ATD. The day 2 scan corresponds to the CSF 5-HIAA nadir. [18F]FCWAY was synthesized by the method of Lang. Dynamic scans were acquired over 2 hours on a GE Advance tomograph (3D mode; 6 mm resolution) following iv. bolus administration of 5 mCi of tracer. IMAGE PROCESSING. Dynamic PET images were motion-corrected, and converted to functional images of distribution volume (DV) using the metabolite-corrected arterial input function. Each subject's MR image was registered to a Talairach template and this transformation was applied to the PET DV images. Cortical and subcortical regions of interest (ROIs), including the raphe, were drawn on structural MR images.
Results: ROI analysis corrected for the free-fraction (f1) parent compound was performed. Comparing the day1 to day 2 scans, we found significant decreases in 5 subjects in 10 brain regions. The largest decrease, 25%, was localized to the raphe (p=0.023). The difference in f1, day1 vs day2, was -11.3% (p=0.012). In the raphe, there was a strong correlation (r=0.823) between day1 and day2 DV data, with intersubject variability being much greater than intrasubject variability.
Discussion: Our data show a measurable effect of ATD on FCWAY/5-HT1A brain receptor binding. There were significant regional decreases in 5-HT1A receptor occupancy, post-ATD. This effect is opposite the hypothesized decrease in synaptic 5-HT/ligand receptor competition. We postulate that acute reduction in 5-HT turnover leads to a compensatory decrease in 5-HT1A auto- and hetero- receptor density. These effects may combine to produce lower FCWAY binding after ATD. It is unclear whether receptor density in other sub-cortical and cortical regions is behaving similarly. However, lower brainstem 5-HT1A receptor density could account for the marked reduction in FCWAY binding seen in the raphe. These findings in healthy subjects may have important implications for the pathophysiology of acute and chronic alcohol dependence.

23. Baseline Data from CTN Study 0010: Buprenorphine/Naloxone Facilitated Psychosocial Treatment for Opioid Dependent Adolescents/Young Adults
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Background: Use of heroin and prescription opioids has increased over the past five years, particularly among adolescents and young adults. Opioid use patterns, prevalence of hepatitis B and C, and HIV risk behaviors have not been well studied among this younger age group. These preliminary data describe these characteristics among opioid dependent patients 14-21 years of age who enrolled in a study comparing the efficacy of outpatient detoxification using buprenorphine/naloxone over 14 days with 12-weeks of buprenorphine/naloxone and counseling.
Methods: 122 patients aged 14-21 meeting DSM-IV criteria for opioid dependence with no serious medical or psychiatric problems other than opioid dependence were enrolled at treatment sites in Albuquerque and Espaniola, NM; Baltimore, MD; Newark, DE; Durham, NC; and Portland, ME. Baseline demographics, opioid use patterns, prevalence of hepatitis B and C, and HIV risk behaviors were examined.
Results: Average age of these 122 patients was 19 years. 78% were 18 or older and 22% were under 18; there were two 15 year olds and none aged 14. The main ethnicity was Caucasian; less than 10% were of Hispanic or African-American ethnicity; 40% were female and 60% male. 74% used heroin >14 days in the past 30 as compared to 5% who used methadone and 26% who used other (prescription) opioids >14 days. Among those who used heroin, average number of days used was 26.4 as compared to 6.7 days for methadone and 15 days for other opioids. Mean years of use were 2.24 for heroin, 3.67 for illicit methadone, 1.7 for prescribed methadone, and 2.5 for other opioids. 22.5% were positive for hepatitis C and 46% for hepatitis B surface antibody. Using injection equipment after another person or using injection equipment to split drug portions was more common among females than males.
Discussion: Prescription opioid use was common; but heroin used by injection was the main drug of abuse. Though using drugs for only about two years, almost 25% had become infected by hepatitis C, probably by sharing injection equipment that was more common among females than males. Efforts to engage these young people in meaningful treatment could have long term benefits in reducing drug use and its complications.

24. A Large-Scale Approach for Identifying Vulnerability Genes to Alcoholism: A 1536 SNP Chip
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Sponsor: Ting Kai Li

Background: A focus on the genetics of alcoholism will identify genes linked to alcohol-specific vulnerability, such as variations in alcohol metabolism, cellular pathways that cause dependence or that are activated during alcohol withdrawal, as well as those linked to shared vulnerabilities such as variation in reward or stress resiliency. However, identifying genetic variants relevant to alcoholism has been hampered by small gene effects, coarse phenotype measurements, and

population admixture. Moreover, due to technological constraints, genetic studies have been limited to the study of small numbers of candidate genes or genetic markers. Here, we propose a large scale approach to study candidate genes for alcoholism by using array genotyping technology.
Methods: We have designed a 1536 SNP panel to identify susceptibility haplotypes in 120 genes implicated in alcoholism as well as addiction in general. Although this panel has been conceived for use on the Illumina genotyping platform, in principle it could be transferred to other technologies. The 120 candidate genes were selected from different domains according to their possible roles in addiction. For example, genes on pharmacokinetic domains involve alcohol metabolism such as ADH and ALDH gene clusters; genes in pharmacodynamic domains include eleven addictive pathways such as the dopamine, serotonin, glutamate, GABA and opioid systems and genes involving stress and behavior discontrol. SNP selection was performed using an automated UNIX based pipeline. The genomic region for each candidate gene (including 5 kb upstream and 1 kb downstream) was retrieved from NCBI Human Genome Build 35.1 (hppt://www.aceview.org). Genotype data for Africans, the population with the greatest genetic diversity, were obtained from HapMap Project. These data were used to estimate linkage disequilibrium (LD) and re-construct haplotype block structures using Haploview (Barret et al, 2005). To maximize the number of genes that could be analyzed, sets of index SNPs that capture all haplotype information using the minimum number of markers, were identified for each gene using a program based on a double classification tree search algorithm (Zhang et al, 2004).
Results: A total of 10.5Mb from 120 genes were processed through this pipeline. The average gene length was 88kb. Within this 10.5Mb of sequence, there were 31,939 SNPs in dbSNP dataset and 4,412 SNP have been genotyped in at least one of four populations by HapMap Project. Using a 0.6% haplotype frequency cutoff, 1,069 index SNPs were selected, with an average of 11.2 SNPs per gene. An additional 267 SNPs which have not been genotyped by HapMap Project but which have been identified as important (functional or potentially functional) were included. Out of the 1336 index SNPs, 247 SNPs were non-synonymous SNP and 75 SNPs were located nearby splicing sites. These SNPs potentially alter gene functions. A total of 200 genomic control SNPs for determination of population stratification were selected for maximum inter-racial frequency differences using HapMap data. Selected SNPs had a minor allele frequency difference (ΔRFA) >0.75 between two populations, and an absolute value of log10 $\geqq$1 for ΔRFA between three populations (ΔRFA1/ΔRFA2) reference allele frequency. The minimum allowable distance between SNPs was 80 kb.
Discussion: Use of this 1536 SNP chip can dramatically reduce genotyping costs, offering the opportunity to study large numbers of candidate genes for alcoholism and addiction. This addictions chip along with supporting bioinformatics data will be made available to all investigators in the field.

25. Nicotinic Agonists Inhibit Reinstatement of Methamphetamine-Seeking Behavior in Rats

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Sponsor: Shigeto Yamawaki

Background: Methamphetamine (MAP) is a highly addictive psychostimulant with reinforcing properties, and the effects are more potent and longer lasting than those of cocaine. Many studies on MAP, like those on other abusive drugs, have focused on mechanisms of the reinforcing effects/drug-taking and sensitization, and, in particularly, very little research has investigated the mechanism of relapse in MAP-seeking behavior. On the other hand, it is suggested that the nicotinic cholinergic system (NCS) in the brain is related to drug addiction and the expression of withdrawal syndrome. In this study, we initially evaluated whether systemic nicotine (NIC) would attenuate the expression of MAP-seeking behavior during the extinction phase in rats self-administering MAP. Furthermore, we clarified the responsible region in the brain concerned with the anti-"craving" effect of NIC, using microinjection methods.
Methods: Male Wistar / ST rats were surgically implanted catheters into the jugular vein in order to self-administer MAP. Guide cannulas were bilaterally implanted into the core of the nucleus accumbens (NAccore), the shell region (NAcshell), the prelimbic (PrC) and infralimbic cortex (InC), the basolateral (BLA) and central (CeA) of the amygdala, and the the ventral (VH) and dorsal hippocampus (DH). Rats were trained to self-administer MAP (0.02 mg/infusion) with a light and tone (MAP associated-cues) under an FR-1 schedule in a daily session for 10 days. Then, extinction sessions under saline infusions without cueing were conducted daily for 5 days. Reinstatement (drug-seeking behavior) tests under saline infusions were carried out on day 6 of extinction and were induced by re-exposure to MAP-associated cues and MAP-priming injection (1.0 mg/kg, i.p.).
Results: Systemic NIC and acetylcholinesterase inhibitor donepezil (DON) attenuate the reinstatement. These inhibitory effects of NIC and DON were blocked by the systemic injection of a nicotinic antagonist mecamylamine (MEC), but not by the muscarinic antagonist scopolamine (SCO). Cue-induced reinstatement was attenuated by microinjection of a local anesthetic lidocaine (Lid) into NAccore, PrC, BLA, CeA and VH and DH, but not the NAcshell and InC, while primed-MAP-induced reinstatement was similarly attenuated by Lid into the same regions except BLA and CeA. Furthermore, microinjection of NIC and DON into the brain regions that showed a positive response to Lid attenuated the reinstatement. The attenuating effects of systemic NIC and DON were reversed by intracranial MEC, but not SCO, into those regions.
Discussion: NIC and DON attenuated the two types of MAP-seeking behavior via NCS in the NAccore, PrC, amygdala and hippocampus. Postmortem clinical study demonstrated that repeated MAP downregulated brain choline acetyltransferase and elevated the expression of vesicular ACh transporter. Judging from these finding, it is suggested that the appearance of MAP-seeking behavior may be related to inactivation of NCS during MAP withdrawal. Furthermore, it is also pointed out that amygdaloid nucleus plays an important role in the appearance of MAP-seeking induced by MAP-associated cue, but not that by MAP-priming injection. Extending the current view on the treatment of drug dependence, our findings suggest that nicotinic cholinergic activating agents may be useful as anti-"craving" agents for preventing reinstatement of MAP-seeking behavior.

26. Diffusion Tensor Imaging Measures Correlate with Cognitive Performance in Heavy Cannabis Smokers

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Background: Evidence for abnormalities in executive function and the ability to successfully monitor and inhibit inappropriate behaviors has been reported in marijuana users and other individuals who abuse illicit substances. Recent investigations which have utilized neuroimaging techniques to evaluate frontal systems in heavy cannabis smokers have reported alterations during inhibitory tasks which require frontal processing. Diffusion tensor imaging (DTI) methods provide a quantitative estimate of white matter integrity, providing new insight at the microstructural level. We applied diffusion tensor imaging techniques (DTI) and a brief neurocognitive battery that included measures of frontal/executive function to examine the relationship between frontal white matter changes and neuropsychological performance in heavy cannabis smokers and matched control subjects.

Methods: Nine heavy cannabis users (age 18-47 years) who had smoked cannabis at least 4000 times in their lives and testing positive for urinary cannabinoids as well as nine healthy, non-smoking control subjects (three or fewer exposures to marijuana) were enrolled in the study. DTI images were acquired on a 3.0 Tesla Siemens Trio scanner using a diffusion weighted twice-refocused spin echo EPI sequence and an eight element receive RF coil. Voxels (11cm3) were manually placed in the midline of the splenium (posterior) of the corpus callosum by a blind rater. Fractional anisotropy (FA), a measure of directional coherence and trace, a measure of overall diffusivity within white matter fiber tracts were calculated in the splenium of the corpus callosum. FA and trace values were calculated from the voxels placed on axial images. Neuropsychological data were acquired on the same day as the scanning sequence.
Results: Similar task performance was found for both subject groups, nevertheless FA of the splenium in marijuana smokers was significantly correlated with both time to complete the interference condition of the Stroop (p=. 029) and the derived Trailmaking score (Trails B minus Trails A; p=. 021). These neuropsychological measures are believed to reflect inhibitory function. No significant correlation was detected in this region for the normal control subjects. Interestingly, higher FA values and lower trace values were associated with better overall performance in the marijuana smokers. No significant relationship was found between urinary cannabinoid concentration and FA or trace, indicating that acute exposure to cannabis is not likely to account for these findings.
Discussion: Data from this investigation provide evidence of altered frontal systems in chronic heavy marijuana smokers, and underscores the importance of examining white matter fiber tracts and their association with inhibitory function in these subjects. The significant association between the FA measure in the marijuana smokers and their performance on neurocognitive measures sensitive to psychomotor function and inhibitory capacity, suggest that exposure to marijuana may result in alterations in white matter fiber tracts. Alterations in these tracts may consequently be related to behavioral outcomes. Supported by NIDA DA 016695 and DA12483.

27. Region and Emotion Specific Effects of Lorazepam on Neural Responses to Facial Displays of Affect

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Background: Lorazepam and other benzodiazepines, widely prescribed and highly effective anxiolytic medications, bind to a specific site on γ-aminobutyric acid (GABA) receptors and are thought to exert their acute effects by enhancing the inhibitory influence of GABAergic neurons. Some emerging evidence suggests that these medications produce region and task specific, dose-dependent changes in neural activation (Sperling et al., 2002; Northoff et al., 2002; Paulus et al., 2005), including general attenuation of affect-related responses in the amygdala and insula (Paulus et al., 2005). However, the specificity of limbic reactivity to negative emotions remains unclear.
Methods: The present study employed BOLD-sensitive whole-brain functional magnetic resonance imaging (fMRI) at 3Tesla (reverse spiral: TR=2s; TE=25ms) to examine the acute effects of lorazepam on brain activation during emotion perception. In a double-blind, placebo-controlled, randomized design, healthy subjects underwent imaging over 2 sessions (at least 1 week apart) and were given either placebo (PBO) or 0.5mg of lorazepam (LZP) intravenously approximately 20 minutes prior to the fMRI behavioral experiment. During fMRI, subjects viewed alternating 20-sec blocks of Ekman Faces expressing discrete emotions (anger, fear, disgust, happy, sad, neutral), interleaved with 20-sec blocks of gray screens. Imaging data were analyzed with a standard, random effects model to determine emotion and drug-specific effects at the group-level using Statistical Parametric Mapping software (SPM2). Thus far, five subjects (3 men, age=37.6±2.9 years) have been studied. Analysis of BOLD signal changes extracted from a priori regions of interest (ROIs) are planned upon completion of the study with additional subjects.
Results: On the PBO day, emotion-specific activations in limbic and paralimbic regions were confirmed; relative to neutral faces, the amygdala (L:[-14,-4,-18], t=4.88, p=0.004; R:[22,-4,-14], t=2.87, p=0.023) was activated bilaterally by faces of fear, while the left insula was activated by faces of disgust ([-34,8,-10], t=8.01, p=0.001). Relative to placebo, LZP attenuated fear-specific activation of the amygdala (L:[-24,0,-24], t=4.47, p=0.006; R:[20,-8,-12], t=4.34, p=0.006) and disgust-specific activation of the insula ([-34,0,-10], t=7.97, p=0.001), in the absence of non-specific effects on primary visual cortex. This pattern was not observed in response to other negative (sadness, anger) or positive (happy) emotional expressions.
Discussion: These preliminary results suggest that the effects of lorazepam can be linked specifically to discrete emotions and localized brain regions. The amygdala and insula have been consistently implicated in anxiety and mood disorders, though benzodiazepines exert variable effects across different psychopathologies. If these findings remain after additional subjects are studied, they could contribute to our understanding of the biologic basis of differential GABAergic mechanisms in mental illness, as well as further substantiate the role of pharmacologic fMRI as a tool for localizing the site(s) of psychiatric drug action in the human brain.

28. Reproducibility of Proton Spectroscopic Imaging (H1-MRSI) at 3Tesla: Effects of Corrections for B1 Field and Partial Volume

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Background: Although much literature exists on the reproducibility of magnetic resonance spectroscopy at 1.5T, little is known about measurement error at higher fields, and no studies exist on the repeatability of H1-MRSI at 3T. Most studies reported coefficients of variation (CV) for repeated scanning sessions between 10 and 20% at 1.5T. Higher field strengths are associated with higher signal but increased susceptibility artifacts and B1 field inhomogeneity degrade the quality of the spectra, making absolute quantitation more difficult. We studied the reproducibility of long time of echo H1-MRSI at 3T.
Methods: 10 normal controls (3 females, ages 18-42) were studied in two scanning sessions 50 days apart on a 3T GE platform. H1-MRSI was obtained with a spin-echo technique (4 slices 7.5mm thick; 11mm gap, acquired on an oblique plane parallel to the hippocampi in the lower slice, nominal voxel volume = 0.42cc, TR/TE 2300/280ms; outer volume suppression pulses for lipid nulling). EPI images at with 11 different flip angles and MPRAGE images were also acquired in register with the H1-MRSI. The EPI acquisition was used to obtain a map of the B1 sensitivity at each voxel. The MPRAGE was used to obtain images segmented into gray, white and CSF compartments. These were then convolved by the point spread function of the scanner in order to obtain the partial volume of each segment for each voxel. ROIs were identified on the fourth slice of the H1-MRSI, corresponding to the gray matter of the right and left dorsolateral prefrontal cortices (rdlpfc and ldlpfc), the medial prefrontal cortex (acing), the white matter of the centrum semiovale (wm) and all the available voxels in the slice (whole). Metabolite values for N-acetylaspartate (NAA), Choline (Cho) and Creatine (Cre) for each ROI were obtained by integration under the peak of the magnitude spectrum and corrected for transmit and amplifier gain, yielding absolute values for the metabolites in arbitrary institutional units. For b1 corrections, each voxel was multiplied by the inverse of the value of the sensitivity map (1 corresponding to an effective 90 deg. flip angle). CSF contribution was accounted for by 1) eliminating voxels with less

than 60% contribution of tissue from the analysis and 2) a regression of the metabolite : % tissue content vs. the % gray matter content : % tissue content. The overall mean of this regression represents the metabolite value after taking into account CSF content. For the ROI called whole the regression model allowed to determine pure gray and white matter values (corresponding to the one and zero intercepts, respectively). The reproducibility of these measures was assessed by the CV. CV values below 10% are usually considered adequate for imaging studies

Results: CVs ranged from 5.9 to 11.6% for raw values, from 3.3 to 9.4% after b1 correction, from 3 to 9.5% after CSF and b1 correction and from 4.8 to 9.1% for gray and white matter determinations in the whole ROI.

Discussion: Several observations are possible from these results: 1) CVs of raw values are usually contained below 10%. Reproducibility of raw values is comparable to that reported at 1.5T and better than most published studies. 2) Correction for b1 inhomogeneity improves CVs for all metabolites. 3) Further correction for CSF content does not seem to modify CVs further. 4) Corrections for pure gray and white matter introduce some additional variability, but they appear to have reasonable reproducibility in a large ROI with a wide assortment of gray and white matter partial volumes. We conclude that if sources of variability are addressed, excellent reproducibility can be obtained at 3T for this type of MRSI acquisition.

29. Neuroticism, Extraversion and Alexithymia Are Associated with Differential Neural Activation Patterns to Emotional Stimuli

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Background: Personality traits such as extraversion, neuroticism and alexithymia have been shown to influence the processing of emotional stimuli and also play a role in the manifestation of specific psychiatric disorders. Recent neuroimaging studies have demonstrated correlations between personality traits such as extraversion and neuroticism and neural activation. However, previous studies that address this relationship have focused on specific emotional stimuli or specialized tasks that limit generalization. In the present study, we examined the influence of extraversion, neuroticism, alexithymia on neural activation patterns to various emotional stimulus types and tasks.

Methods: To address this question, we analyzed the combined data from three independent studies (n=38) involving passive viewing or appraisal of emotional stimuli. All healthy participants completed the self-administered NEO-PI-R (to derive neuroticism and extraversion scores) and the TAS-26 (to derive alexithymia scores). Within each study and for each individual, linear contrasts investigated the effects of positive and negative emotional content, isolating emotional activation. Indices of neuroticism, extraversion, and alexithymia were correlated with activations to positive and negative stimuli by entering these scores as covariates of interest in separate SPM analyses. Regions-of-interest analyses further confirmed the correlational relationship between activation and personality traits.

Results: Neuroticism scores inversely correlated with extraversion scores ($R2 = -0.338$, $p<0.038$) in the combined large group of subjects (n=38). Neuroticism scores correlated with BOLD response in rostral dorsomedial prefrontal cortex [(12, 54, 21), Z=3.14, k=13] in response to positive relative to neutral stimuli. Extraversion scores inversely correlated with BOLD activation in bilateral anterior insula [left: (-33,18,6), Z=2.67, k=7; right: (33,27,-9) Z=2.91, k=14] in response to negative relative to neutral stimuli. Finally, in response to positive and negative films, alexithymia scores directly correlated with posterior insula activation [positive: (-42,-12,12), Z=3.96, k=32; negative: (-42,-12,-6), Z=3.30, k=9].

Discussion: Our findings suggest that in a healthy population personality traits (neuroticism, extraversion and alexithymia) may influence neural activation patterns during emotional processing. Higher neuroticism scores were associated with higher dmPFC activation to positive stimuli, possibly reflecting exaggerated self-relatedness processing. Higher extraversion scores were associated with lower insula activation to negative stimuli, possibly reflecting increased sensitivity to negative stimuli in individuals with low extraversion scores. Higher alexithymia scores were associated with higher insula activation to emotional films, possibly reflecting an increased somatic emotional processing when recalling emotional events. Future studies are needed to replicate these findings and examine their relevance in patient populations.

30. Magnetic Resonance Spectroscopic Imaging Twenty Years After the Emergence of Adolescent-Onset Major Depressive Disorder

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Background: Adolescent-onset major depressive disorder (MDD) imparts substantial adult morbidity and risk of mortality from suicide. The present study sought to determine regional brain concentrations of the neurometabolite N-acetyl-aspartate (NAA) in adults diagnosed approximately 20 years earlier with adolescent-onset major depressive disorder (MDD). NAA concentrations, which correlate with metabolic activity and tissue injury, might distinguish these patients.

Methods: The adolescent-onset MDD group was originally recruited between 1977-1985 at Columbia-Presbyterian Hospital, and 73 members of this cohort were followed-up by Weissman between 1992-1996. Twenty-one subjects were located for the current study, which was conducted between 2002-2004. Twelve declined participation. The remaining nine patients (mean age= 35.4 [2.2]; 5M/4F; four on psychotropic medication at the time of scan; mean age of onset of MDD=14.0 [1.6]; all right-handed) underwent proton magnetic resonance spectroscopic imaging to determine NAA concentrations in 14 brain regions. Nine comparable healthy adults served as control subjects. Scans were performed on a 1.5 T GE Signa MR system using a standard multi-slice H-MRSI method. A clinically experienced interviewer, blind to previous evaluations, conducted a SADS interview to assess lifetime psychopathology, and a study psychiatrist performed psychiatric rating scales prior to the scan.

Results: Principal follow-up diagnoses included MDD (5/9), bipolar I or II (3/9), and OCD (1/9). The number of diagnoses per patient ranged from 1 to 5 (median=3). Severity of depressive and anxiety symptomatology varied greatly: median Hamilton Rating Scale for Depression-24 score =16 (range= 1-27); median Hamilton Anxiety Scale score=16 (range 1-22). Two females (1 medicated, 1 unmedicated) were remitted and well-functioning at the time of scan. In patients' dorsolateral prefrontal cortex (DLPFC), NAA concentrations were lower in the right hemisphere compared to the left, while the opposite pattern occurred in comparison subjects. This was quantified by a laterality index ([left NAA - right NAA]/[left NAA + right NAA], which differed significantly between patients and comparison subjects ($t=2.92$, $df=16$, $p=.010$). Similarly, in hippocampus, substantially greater relative right hemispheric concentrations of NAA characterized patients in remission compared to symptomatic patients ($t=5.26$, $df=7$, $p=.001$). Absolute NAA concentrations did not differ between patients and comparison subjects in any region assessed.

Discussion: Psychiatric morbidity subsequent to adolescent-onset MDD continues well into adulthood. In addition, neuroimaging revealed that direction of hemispheric laterality of NAA in DLPFC is a possible marker for susceptibility to or history of adolescent-onset MDD. These data are consistent with previous findings documenting stable assymetric frontal EEG patterns of activation related to emotion, in which increased right-hemispheric activity correlates with

negative affect. Prospective diagnostic assessment of patients in the current study at three timepoints beginning in adolescence and continuing over twenty years permitted more precise evaluation of age at MDD onset and of course of illness than would otherwise have been possible. However, the small number of patients recruited from the original sample may limit the generalizability of these findings.

31. COMT val^{158}met Polymorphism Impacts on Reactivity of Amygdala to Emotion Laden Stimuli

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Background: Converging evidence indicates that the val^{158met} polymorphism in the gene for catechol-O-methyl transferase (COMT), an enzyme that degrades catecholamines, accounts for individual variability in executive function as well as mood related behaviors. In this study, using event-related fMRI, we explored the effect of this genetic polymorphism on the amygdala reactivity during a task in which the subjects had to decide whether they would approach or avoid emotionally laden facial stimuli
Methods: Twenty-six normal subjects [9 val/val (3 males, age±+SE, 32 ± 6), 8 val/met (2 males, age±SE, 30 ± 9) and 9 met/met (4 males, age±SE, 28±8)], who had undergone extensive clinical evaluation, were recruited. Genotype groups were matched for age, IQ, handedness and sex. FMRI was performed on a 3 Tesla GE scanner using gradient echo EPI while subjects performed an event-related paradigm during which they had to respond whether they would ″approach″ or ″avoid″ facial stimuli (Angry, Fearful, Neutral and Happy NimStim/Macarthur faces). Image analysis was performed using the general linear model in SPM 99.
Results: In general, as expected, we found that there was greater amygdala reactivity during ″avoid″ relative to ″approach″ stimuli, particularly for fearful and angry faces. Most interestingly, we found that the COMT val^{158}met polymorphism also affects the functional reactivity of the amygdala: individuals homozygous for the low-activity met allele showed greater amygdala activity than val carriers ($p<0.01$). This response was particularly greater during the ″avoid″ stimuli.
Discussion: These findings lend support to the existing literature that the low activity met allele contributes to decreased resilience against negative mood states, such as anxiety and dysphoria, by biasing amygdala reactivity.

32. Irritable Bowel Syndrome and Increased Anxiety in Healthy Females, but Not in Healthy Males, is Associated with Increased Size of a Midbrain Region Activated During Inescapable Pain

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Background: The ventrolateral column of the midbrain periaqueductal grey (vlPAG) has been functionally associated with visceral and inescapable pain. Both presence of Irritable Bowel Syndrome (IBS) and high levels of anxiety are associated with pain hypersensitivity. We have also reported increased PAG activation by visceral distention in patients with IBS compared to controls.
Methods: Subjects with and without constipation predominant IBS (matched for age) were imaged with a 1.5T Siemens Sonata MRI camera using an MP-RAGE pulse sequence to produce 3-D T1 weighted images having resolution of 1.0 cubic mm that covered the entire brain. Optimized voxel based morphometry was conducted on normalized images after registration into a common atlas space. Statistical Parametric Mapping (SPM2) was used to make voxelwise group comparisions. Covariance analyses assessed the relationship between anxiety and size of vlPAG. A second sample of male and female healthy control subjects were studied in a similar fashion using a 3T Siemans Allegra scanner.
Results: vlPAG was significantly larger in female patients with constipation-predominant IBS (n=11) as compared to healthy controls (n=10) ($p<.007$). In addition, in the healthy females ($r=.90$, $F=33.1$, $p=.0004$), but not in the IBS patients ($r=-.41$,NS) the size of vlPAG was positively correlated with state anxiety measured by validated questionnaire (Hospital Anxiety Questionaire). This may be due to restricted range since the IBS patients had higher anxiety levels (although none had anxiety disorder). In a separate sample of healthy males and females using the same methods, trait anxiety (State Trait Anxiety Inventory) was positively correlated with vlPAG in 4 healthy females ($r=.93$, $F=13.0$, $p=.069$), but not in 9 healthy males ($r=.35$, NS).
Discussion: Association of anxiety with vlPAG size in two samples of healthy females suggests females with larger vlPAG may be predisposed to higher anxiety. It may also be that Female IBS patients show increased vlPAG due to higher anxiety levels. Recent demonstration of reversible plasticity in the size of circumscribed brain regions with learning suggest an alternative hypothesis, namely that chronic anxiety itself may increase the size of vlPAG. The gender finding may relate to the female preponderance in functional pain disorders, suggesting a possible structure-function sensitization restricted to females. Supported by NIH grants: P50 DK64539 (EAM), DK 48351 (EAM), NR 04881 (BN), and R24 AT002681 (EAM).

33. Increased Basal Ganglia Activity During Interferon-Alpha Therapy for Malignant Melanoma: Relationship with Neurovegetative Symptoms

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Background: Administration of the cytokine, interferon (IFN)-alpha, is associated with behavioral co-morbidities, including depression, cognitive dysfunction and neurovegetative alterations such as fatigue, anhedonia and motor disturbance. While recent advances have been made in characterizing the pathophysiological mechanisms underlying IFN-alpha-induced mood/cognitive symptoms, those underlying neurovegetative symptoms remain to be elucidated.
Methods: The present study assessed the role of the basal ganglia in the pathophysiology of IFN-alpha-induced neurovegetative symptoms using brain positron emission tomography in twelve patients with Stage II-IV malignant melanoma devoid of metastatic disease. Regional cerebral glucose metabolism in basal ganglia was estimated by fluorine-18-labeled fluorodeoxyglucose uptake under resting conditions at baseline (before IFN-alpha administration) and after four weeks of IFN-alpha therapy. Before each scanning session, neuropsychiatric symptoms were evaluated.
Results: Neurovegetative symptoms, including tiredness/fatigue, inability to feel/anhedonia and motor alterations significantly increased during IFN-alpha therapy. In addition, IFN-alpha therapy was associated with significant and widespread bilateral increases in glucose metabolism in all areas of the basal ganglia, aside from the caudate nucleus where increases were only significant on the right side. Increased glucose metabolism in the globus pallidus and in the right caudate nucleus correlated respectively with the development of IFN-alpha-induced tiredness/fatigue and inability to feel/anhedonia.
Discussion: These findings suggest that changes in basal ganglia activity during IFN-alpha therapy play a role in the pathophysiology of neurovegetative symptoms. In addition, the data suggest that basal ganglia activation secondary to endogenous cytokines released dur-

ing cancer or its treatment may be involved in neurovegetative symptoms in other populations of cancer patients.

34. Distribution of [11C]PIB in a Nondemented Population: Implication for Use as an Antecedent Marker of Alzheimer's Disease

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Background: Neurofibrillary tangles and beta-amyloid (Aβ) plaques are the pathological hallmark of Alzheimers disease (AD). Recently, a PET imaging tracer that binds to Aβ plaques *in vivo*, N-methyl-[11C]2-(4'-methylaminophenyl)-6-hydroxybenzothiazole (or [11C]PIB for "Pittsburgh Compound-B"), has been shown to have significantly higher binding in subjects diagnosed with AD compared to nondemented controls. We have previously proposed that Aβ plaques exist in AD prior to the onset of dementia and are now examining whether [11C]PIB can be used to detect this "preclinical" state.
Methods: 41 nondemented subjects (65.3 ± 16.1 yrs) with Clinical Dementia Rating (CDR) values of 0, and 10 demented subjects (77.8 ± 6.6 yrs) of the Alzheimer's type (DAT) with CDR values of 0.5 or 1, underwent [11C]PIB PET scanning. Dynamic PET scans (60 min) were corrected for head movement and registered to a 3D T1-weighted MRI image. Regions-of-interest (ROIs) were drawn on the MRI over the cerebellar, prefrontal, lateral temporal, occipital, gyrus rectus, precuneus, and striatal cortex. After extraction of the PET time-activity data, Binding Potential values (BPs), proportional to the density of [11C]PIB - Aβ binding sites, for each ROI were calculated using the Logan graphical analysis and the cerebellar cortex for a reference tissue. The BP values for the gyrus rectus, prefrontal, lateral temporal and precuneus cortex were averaged to create a mean selected cortex BP (scBP).
Results: DAT subjects had significantly ($p < 0.0001$) elevated scBP values (mean scBP = 0.63 ± 0.35) compared to the entire group of nondemented subjects (0.05 ± 0.16) and to an age-matched subset (77.4 ± 5.2 yrs) of 20 nondemented subjects (0.09 ± 0.24). Interestingly, one of the 10 DAT subjects (CDR = 0.5) had a scBP value of essentially zero (scBP = -0.05) and visually had no increased [11C]PIB retention. Of the 41 nondemented subjects there were four subjects with elevated scBP values (subject ages 61, 72, 74 and 77 yrs). The mean scBP of these four subjects (0.47 ± 0.26) was somewhat lower than the mean of the DAT subjects but was not significantly different ($p > 0.4$). Inspection of the [11C]PIB images and individual ROI BP values revealed two of these four nondemented subjects had [11C]PIB uptake, both visually and quantitatively, that was indistinguishable from the DAT subjects. However, in the other two nondemented subjects the increased uptake seemed most elevated in the precuneus with less uptake in temporal and prefrontal cortices.
Discussion: These results replicate and extend the findings of Klunk et al (2004) demonstrating elevated [11C]PIB binding in subjects with DAT compared to nondemented controls. This body of data suggests that [11C]PIB amyloid imaging does strongly correlate with the clinical presence of AD. Even the single DAT subject without elevated [11C]PIB binding is not unexpected given the known limitations of early clinical diagnosis of AD. The findings of elevated [11C]PIB binding in four nondemented subjects, while preliminary, suggest that [11C]PIB amyloid imaging may be sensitive for detection of a preclinical AD state. Longitidunal studies will be required to determine the association of elevated [11C]PIB binding and future risk of developing DAT. Finally, the apparently selective elevation of the [11C]PIB binding in the precuneus in two nondemented subjects raise the possibility that the precuneus may be a critical early area of pathological involvement in AD.

35. Positron Emission Tomography of Brain 5HT1A Receptor Binding with [11-C]WAY100635 in Postpartum Depressed and Nondepressed Women

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Brain serotonin-1A (5HT1A) receptor density, mRNA expression, and function are reduced in major depressive disorder, as demonstrated by post-mortem, in-vivo positron emission tomography (PET), and neuroendocrine studies. The reproductive and placental hormones estradiol and cortisol have been shown to reduce 5HT1A receptor density in experimental animals. We hypothesized that postpartum depression (PPD) would be accompanied by a reduction of 5HT1A receptor BP due to combined vulnerabilities of hypercortisolemia, long-term hyperestrogenemia during pregnancy, and depressive illness.
Methods: Five unmedicated PPD (4 bipolar; 2 breastfeeding; mean age 28) and five postpartum control (PPC; 4 breastfeeding; mean age 32) subjects underwent 90-min dynamic positron emission tomography (PET) using a Siemens/CTI HR+ and i.v. administration of [11-C]WAY100635 (15 mCi). Logan graphical analysis was used to derive 5HT1A receptor binding potential (BP) for mesiotemporal cortex (MTC), lateral orbitofrontal cortex (LOF), pregenual cingulate cortex (PRE), subgenual cingulate cortex (SUB), and raphe nucleus (RN), with cerebellum (CER) as the reference region. BP was calculated as $[(DV_{ROI}/ DV_{CER}) - 1]$, where DV is the distribution volume.
Results: Age, duration postpartum, BMI, progesterone, prolactin and cortisol concentration did not differ between groups. Mean estradiol concentration was higher in the PPD (47.2 ± 16.2 pg/ml) relative to the PPC group (25.0 ± 9.7 pg/ml). Logan graphical analysis revealed a 20 to 26% reduction of 5HT1A receptor BP in the PPD versus PPC group in MTC, PRE, and SUB [p(MWU; 2-tailed;exact) = 0.02). There were no significant group differences in 5HT1A receptor BP in LOF or RN. CER DV was not significantly different between groups. When we removed an outlier from the PPD group, the findings remained significant, and CER DV of the PPD group was more similar to that of the PPC group. BP derived with 2-tissue compartmental modeling was correlated with Logan-derived BP in MTC, RN, LOF, PRE and SUB (R = 0.65 to 0.83; p = 0.01 to 0.06).
Discussion: These preliminary findings demonstrate that postsynaptic 5HT1A receptor BP is reduced in PPD versus PPC women by a similar magnitude as shown previously in non-postpartum depressed versus control samples. The neurobiological similarity between this PPD sample and non-postpartum depressives is not surprising given the similarity in clinical phenomenology and antidepressant treatment response. It remains unclear whether the hormonal milieu at 2 to 3 months postpartum and the large proportion of bipolar subjects in the PPD group accentuated this finding in this small sample. Support: MH64561

36. The Sensitivity of the Loudness Dependence Auditory Evoked Potential (LDAEP) as a Measure of Central Serotonin Function in Humans

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Sponsor: Matthew Galloway

Background: The Loudness Dependence of the Auditory Evoked Potential (LDAEP) has been suggested as a possible invivo measure of central serotonin function. The LDAEP is a measure of auditory cortex activity, reflecting an increase or decrease in the slope of auditory evoked potentials (N1/P2) with increasing tone loudness. The most

convincing evidence for a direct relationship between serotonergic function and LDAEP to date has come from animal studies, while evidence in humans has been circumstantial and inconsistent
Methods: We directly examined the acute and chronic effects of serotonergic modulation with the serotonin reuptake inhibitors (SSRIs) citalopram (20mg; acute at 2h post drug; n=11) and sertraline (50mg; chronic, 21 days; n=33) on the LDAEP in healthy subjects. The studies were double blind and placebo controlled with subjects tested in a repeated measures design for the acute study and an independent group design for the chronic study. Tones were presented at intensities, 60, 70, 80, 90 and 100 dB. The magnitude of the N1 and P2 peaks were determined for each intensity at Cz. The slope of the N1/P2 was calculated as the linear regression slope with stimulus intensity as the independent variable and N1/P2 amplitude (calculated as P1 minus N2) as the dependant variable
Results: Acute enhancement of serotonin levels with citalopram in comparison to placebo decreased the slope of the LDAEP (i.e. weaker LDAEP) ($p = 0.006$). Similarly chronic enhancement of serotonin levels with sertraline in comparison to placebo, decreased the slope the LDAEP ($p=0.04$).
Discussion: These findings provide direct evidence in humans for a robust relationship between changes in central serotonin levels and the LDAEP, supporting findings previously observed in animals and clinical populations. Together the results provide further support for the validity of the LDAEP as a non-invasive invivo measure of central serotonin function in humans.

37. FDG-PET in Laboratory Induced Aggression in Borderline Personality Disorder

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Sponsor: Larry Siever

Background: Patients with borderline personality disorder (BPD) frequently suffer from impulsive aggression (IED), which can pose a serious threat to themselves and to others. Preclinical studies suggest that the orbital frontal cortex (OFC) and anterior cingulate gyrus (ACG) play an inhibitory role in the regulation of impulsive aggression. Our previous PET studies employing the serotonin agonist, meta-chlorophenylpiperazine, showed that BPD patients with IED exhibit less activation in the OFC and ACG than healthy controls. The present study extends this line of research by using ^{18}FDG-PET scans during an anger provocation task, the Point Subtraction Aggression Paradigm.
Methods: 16 controls (9 male, 7 female) and 14 age-matched BPD-IED patients (10 male, 4 female) completed the PSAP during FDG uptake. All subjects underwent two PET scans, one while performing an aggression provocation task and the other during a control non-provocation task, conducted in a counterbalanced manner. Brain edges were visually traced on MRIs, which were coregistered with PET scans and relative glucose metabolic rate (rGMR) was obtained for 40 cortical Brodmann Areas (BAs) bilaterally. Difference scores (no provocation minus provocation condition) for rGMR in the OFC and ACG were evaluated.
Results: For the ACG, rGMR was entered into a mixed-design ANOVA consisting of group (BPD, NC) x provocation level (provocation, no provocation) x hemisphere (R, L) x BA (25, 24, 31, 23, 29). A significant group x provocation condition interaction was detected ($F(1,28)=4.30$, $p=0.048$, Wilks) showing increased cingulate activity to aggression provocation in controls, and decreased cingulate activity in response to aggression provocation in patients across the arc of the cingulate gyrus. For the OFC, rGMR was entered into a diagnostic group (BPD, NC) x provocation level (provocation, no provocation) x hemisphere (R, L) x BA (11, 12, 47) mixed-design ANOVA. The main effect of diagnostic group and a group x hemisphere interaction were significant, but none of the interactions with provocation level were significant. This indicates baseline decreased OFC activity in BPD-IED patients compared to controls.
Discussion: BPD-IED patients show decreased activity in the cingulate gyrus in response to aggression provocation, while healthy controls show increased cingulate activity in response to anger provocation. These findings give further support to the model suggesting that underactivation of the cingulate gyrus, a brain region critical to the modulation of aggressive responses, may underlie that emergence of aggression in borderline personality disorder.

38. Acute Occupancy of Brain Serotonin Transporter by Sertraline as Measured by [11C]DASB and Positron Emission Tomography

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Sponsor: J. John Mann

Background: In vivo determination of serotonin transporter (5-HTT) occupancy by selective serotonin reuptake inhibitors (SSRI) using positron emission tomography (PET) can aid in determination of dosing. Previous studies have investigated chronic SSRI administration that may down-regulate 5-HTT and used the cerebellum as reference region despite measurable 5-HTT. We examine the reference region and occupancy after acute sertraline dosing.
Methods: We conducted autoradiography of human postmortem cerebellum to determine an optimal reference region. We quantified 5-HTT binding using [11C]DASB and arterial input functions in 17 healthy volunteers. Baseline PET scans were followed by a scan 4-6 days after 25 mg to 100mg of daily sertraline. Plasma sertraline was measured at the time of the occupancy PET scan. The impact of several modeling methods and outcome measures was assessed.
Results: Cerebellar gray matter was determined to be the optimal reference region. Occupation of 5-HTT sites saturates at low plasma sertraline levels (KD = 1.9 ng/ml) with maximal occupancies of 106.8 *plusmn* 8.3% across all brain regions. There is a weak correlation between oral sertraline and plasma sertraline. Occupancy measures vary based on the reference region and outcome measure used.
Discussion: Occupancy studies and postmortem autoradiography can help define the optimal reference region. Reference tissue modeling returns the same occupancy measures as those determined using an arterial input function.

39. Lorazepam Dose-Dependently Decreases Risk-Taking Related Activation in the Caudate

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Sponsor: Murray Stein

Background: Risk-taking decision-making is a complex process and is fundamentally related to the anticipation of aversive outcomes. Thus, it is not surprising that some investigators have linked anxiety to reduced risk-taking. Previous neuroimaging investigations have shown that a distributed neural system involving the anterior insula, striatum, dorsolateral prefrontal cortex and posterior parietal cortex may be important in computing risk assessment. Moreover, the degree of activation in the anterior insula during risk-taking decision-making was associated with the level of neuroticism and harm avoidance. This investigation was aimed to clarify the role of GABAergic modulation of neural substrates underlying risk-taking decision-making.
Methods: 14 healthy volunteers participated in a double-blind, placebo controlled, randomized dose-response study. Subjects were scanned three times (at least a week apart) and given a single-dose placebo, 0.25 mg or 1.0 mg lorazepam one hour prior to an MRI session. During fMRI, subjects completed risk-taking decision-making task. The main outcome measure was the BOLD-fMRI activation during risky decision-making relative to selecting a safe response.

Results: Lorazepam dose-dependently decreased the selection of a risky response after a punished trial but not after a non-punished trial ($F(2,26) = 3.86$, $p < 0.05$). Moreover, there was a dose-dependent decrease of activation in caudate, dorsolateral prefrontal cortex and posterior parietal cortex but not in the anterior insula. The dose-dependent decrease in the caudate was correlated with the decrease in risky responses after punishment.
Discussion: This investigation shows that GABAergic manipulation attenuates risky responses after punishment, the degree of which is associated with an attenuation of the caudate. However, there was no dose-dependent effect in the anterior insula. In a previous study, using an emotion face-processing task, we found dose-dependent attenuation in the amygdala and anterior insula. In combination, these results support the general hypothesis that the pharmacological effect of lorazepam on specific neural substrates depends on the nature of the task at hand. Moreover, the task-specific attenuation of neural substrates argues against an unspecific attenuation of the fMRI-BOLD response.

40. An fMRI Study of the Interface Between Affective and Cognitive Circuitry in Pediatric Bipolar Disorder

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Sponsor: Ghanshyam Pandey

Background: In this study, we investigated the impact of affective challenge on cognitive function in pediatric bipolar disorder, given the close anatomic and functional connectivity between affective fronto-limbic (orbitofrontal cortex (OFC); ventral cingulum; amygdala) and cognitive fronto-striatal systems (dorsolateral prefrontal cortex (DLPFC); posterior cingulate; caudate; putamen).
Methods: In an fMRI study, ten unmedicated euthymic bipolar type I subjects were compared with ten age (12-18 years) and gender matched healthy controls (HC). The task consisted of an affective Stroop paradigm, aiming to recruit attention under emotional challenge.
Results: In the negative word condition, relative to the neutral word condition, bipolar subjects displayed increased activation in the amygdala, ventral anterior cingulate gyrus, insula and caudate. In comparison, HC showed increased activation in posterior cingulate gyrus and DLPFC. In the positive word condition, bipolar patients showed activation in the nucleus accumbens, while the HC displayed activation in DLPFC, posterior cingulate gyrus, and putamen.
Discussion: Our findings suggest that matching negative affect in bipolar individuals leads to over activation of affective circuitry at the expense of attentional systems, while HC are less affected by emotional challenge and were able to better engage cognitive circuitry in the context of emotional arousal. When matching positive words, the reward based nucleus accumbens was engaged in bipolar subjects while HC have differentially recruited the cognitive system. These findings have direct clinical implications for pediatric bipolar disorder in suggesting that emotional disturbances may contribute to cognitive processing deficits in the disorder.

41. FDG and PIB PET Imaging in Alzheimer's Disease and Mild Cognitive Impairment

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Background: Previously we reported quantitative positron emission tomography (PET) imaging studies of the amyloid-binding radiotracer, Pittsburgh Compound-B (PIB) in patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI) and healthy control (HC) subjects. These studies showed clear distinction of cortical PIB retention in AD and HC subjects, MCI PIB retention that ranged from control to AD levels, similar non-specific PIB retention across groups, and similar findings whether cerebellar data or arterial data were used in the analyses. The present work reports on our initial efforts to relate PIB retention to metabolism (via FDG PET) across AD, MCI, and control subjects.
Methods: PET imaging was performed (ECAT HR+ scanner) for 8 mild-to-moderate AD (MMSE=25±3; 68±8 yrs), 10 MCI (MMSE=27±1; 72±9 yrs), and 12 control (MMSE=30±1; 68±13 yrs (n=10), 39 yrs, and 45 yrs) subjects. PIB and FDG imaging were performed the same day (PIB: 15 mCi, 90 min; FDG: 7 mCi, 35-60 min post-injection). Magnetic resonance images were acquired for region-of-interest (ROI) definition and atrophy correction. ROIs included posterior cingulate gyrus (PCG), parietal (PAR), frontal (FRC), lateral temporal (LTC) cortices and cerebellum (CER). PIB data were analyzed using the Logan graphical method with cerebellar data as input (distribution volume ratio, DVR). The FDG analysis was based upon late summed images (40-60 min post-injection) and the resulting ROI measures were normalized to the CER value (uptake ratios). The PIB DVR and FDG uptake ratio measures were compared on an ROI basis using 1) Spearman correlations and 2) a 2-block partial least squares (PLS) approach with singular value decomposition of the cross-block covariance. The PLS analysis provided an overall score for either PIB or FDG that was calculated across ROIs for each subject (based upon the first and primary component).
Results: Relative to controls, the AD subjects exhibited significantly lower ($p<0.025$) FDG metabolic ratios in PCG, FRC, and LTC areas, while AD PIB retention was significantly greater ($p<0.025$) in these same areas (also in mesial temporal and occipital cortices). Cortical PIB retention was clustered for AD and HC subjects (e.g., PCG: AD DVR = 2.36±0.26, HC DVR = 1.19±0.11). No significant correlation was found between the FDG and PIB ROI outcomes for the AD subjects, although a significant positive correlation was observed for the LTC region of controls ($r = 0.61$, $p=0.03$) with a positive trend in FRC. The PLS analysis yielded FDG scores that ranged across 1.5 units without clear separation of the AD and HC scores with MCI scores that ranged across AD and HC levels. The PLS PIB scores exhibited a greater dynamic range (across 5.0 units) with clear separation of the AD and HC groups. The PIB scores clustered into a "control+MCI" group, an intermediate group that contained 3 MCI subjects, and a third "AD+MCI" group.
Discussion: The AD brain areas for which reductions in FDG uptake were most significant also exhibited significant increases in PIB retention, as compared to controls. The PIB PLS scores clearly separated mild-to-moderate AD subjects from controls according to clinical diagnosis with a dynamic range that allowed for an intermediate retention level that contained only scores from the MCI group. This level of discrimination was not achieved using the PLS FDG scores for this small sample. (This work was supported by MH070729, NIA, DANA Foundation, GE Health Care)

42. Imaging Neuroinflammation in Alzheimer Disease with [1-11C] Arachidonic Acid and Positron Emission Tomography

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Background: Arachidonic acid (AA), a component of membrane phospholipids, is released by phospholipase A2 (PLA2) activation. Neuroinflammation, which is considered to contribute to AD, can increase PLA2 activity and AA turnover in brain phospholipids; this process has been imaged in unanesthetized rats (Lee, JNeurochem, 91, 936, 2004).
Methods: Regional brain AA incorporation coefficients (K^*) were measured with [1-11C]AA and positron emission tomography (PET)

(Giovacchini, JCBFMetab, 22, 1453, 2002) in 8 moderately-severely demented AD patients and 7 age-matched controls; regional cerebral blood flow (rCBF) was measured with 15O-water. PET images were corrected for partial voluming from MRI scans. Data were normalized to a global mean, which was independently determined. SPM2 was used to test for group differences. Results are at $p < 0.001$.
Results: Compared to controls, rCBF reductions of ~21% were in temporal (BA 37, 20 and 22) and 20-40% in parietal (BA 39 and 7) association cortex bilaterally of AD patients. AD patients had increased normalized K* for AA of ~15% in temporal association cortex bilaterally (BA 21 and 22), ~12% in the right parietal lobule (BA 39), and 10-14% bilaterally in orbitofrontal cortex (BA 11 and 47). Between group differences were greater with absolute K* and rCBF values. Compared to controls, AD patients had 35% decreased mean global CBF and 23% increased global K*.
Discussion: AA incorporation is increased in AD compared with control brainn, globally and regional in brain areas demonstrating inflammatory neuropathology on postmortem; rCBF reduddctions in these areas are consistent with prior evidence of reduced neuronal activity. As animals studies indicate that K* increases represent neuroinflammation involving increased PLA2 activity and AA turnover (Rosenberger, JNeurochem, 88, 1168, 2004), our findings suggest that neuroinflammation can be imaged in vivo in AD patients. PET imaging of AA incorporation might be used as a surrogate marker of AD neuroinflammation, for early diagnosis and evaluation of disease progression.

43. A Voxel-Based, Whole Brain MRI Analysis of Adolescents with Psychotic Illnesses

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Sponsor: Martin Reite

Background: Structural neuroimaging-based studies of psychosis generally focus on adults with on schizophrenia (Sz), schizoaffective disorder (SzA), and psychotic mood disorders (PMD), and are generally limited to examination of a small number of manually-traced structures. Voxel-based morphometry (VBM) provides a highly automated and standardized approach to quantifying group differences in brain structure. Although VBM has been applied to the study of psychosis in adults, childhood-onset psychosis has not been widely investigated using this methodology.
Methods: 1.5T MRI data were acquired for 11 adolescents age 14.6 +/- 1.9 years (range 10-17) with onset of a psychotic illness prior to age 13 years (Sz+SzA n = 5; PMD n = 6). Identical scans were obtained among eleven age and gender matched adolescents with no history of mental illness. An optimized VBM approach implemented in SPM2 was employed. All subjects underwent assessment of general intellectual functioning and were examined for neurological soft signs using the Neurological Evaluation Scale (NES).
Results: No differences in overall gray matter, white matter, or total brain volume were observed between the two groups. After controlling for total volume and IQ using ANCOVA, significantly larger gray matter volumes were observed in the left temporal pole (Brodmanns area (BA) 38) and left anterior cingulate gyrus (BA32) among subjects with psychosis. Larger gray matter volumes were also observed in the right temporal pole (BA38). Smaller gray matter volumes were observed in the left hemisphere of the subjects with psychosis, including the cingulate gyrus (BA24), superior parietal lobule (BA 7), postcentral gyrus (BA 3), superior temporal gyrus (BA 22) extending to the insula, pre-central gyrus (BA 4), middle frontal gyrus (BA 13), medial frontal gyrus (BA 6), superior frontal gyrus (BA 6), and parahippocampal gyrus (BA 30). With respect to white matter, the right middle cerebellar peduncle was significantly smaller in the psychosis group. To analyze relationships between brain structure and neurological signs, NES, IQ and diagnosis were entered as independent variables in a multiple regression analysis. After controlling for diagnosis and IQ, significant partial correlations between NES and the residual variance in gray matter were observed in the left frontal lobe (BA 4, 6 10 and 24), left occipital lobe (BA 18 and 19), left parietal lobe (BA 7 and 40), left temporal lobe (BA 22 and 37), and left insular cortex (BA 13). In the right hemisphere, significant partial correlations were observed in frontal (BA 8 and 45), parietal (BA 7, 19 and 40), temporal (BA 39), insular cortex (BA 13) and putamen.
Discussion: The present findings largely replicate in adolescents with psychotic disorders regional reductions in gray matter volume observed in adults with these conditions. Additionally, severity of neurological soft signs correlated with larger gray matter volumes in several bilateral regions responsible for motor control, attention and planning. These findings suggest that aberrant neurodevelopment in adolescents may be reflected by alterations in regional cerebral volumes. The correlation with NES scores suggests that the volumetric alterations may be functionally significant. The contrast between temporal pole findings in the present and prior studies suggests that findings of volumetric reductions in adults with psychotic disorders may, in some regions, be preceded by abnormally large volumes earlier in these illnesses. Further studies are needed to replicate the present findings and to investigate the timing of neurodevelopment abnormalities among persons with psychotic illnesses.

44. Localization of Increased White Matter Hyperintensities in Late Life Depression Using an Automated Voxel-Wise Method

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Background: White matter hyperintensities (WMH) are commonly seen on T2-weighted MRI and are associated with age, cognitive loss and depression. Increased WMH in elderly depressed subjects has been a well-replicated finding. However, WMH are also noted to occur at rates of up to 60% in healthy elderly patients in whom their significance is unknown. In addition to the overall number of WMH the site of the lesions may be important in predicting vulnerability to depression and the cognitive deficits associated with late life depression. Our goal in the current study was to systematically identify and compare WML location and volume between depressed and control subjects.
Methods: Late-life (age $\geqq$ 60) depressed subjects (n=91) and matched controls (n=21) were imaged using a Siemens Sonata 1.5T MRI scanner to acquire T2W and 3D T1W data: The T1W scans were coregistered to correct for head motion prior to averaging. The TSE scans were coregistered with the T1W scans and both were transformed into Talairach space. Parabolic gain field corrections were performed on both images to allow whole brain tissue type determination. A 2D histogram of pixel values was used to identify peaks for CSF, air, gray matter, white matter and WMH. Segmented image by tissue type was created by class assignment based on nearest tissue-type locus in a bi-spectral histogram. From these segmented images of tissue types, the volume and location of WML was projected into atlas space for statistical comparison. First the segmentation difference volumes were smoothed (10mm FWHM Gaussian profile). Then using a combination of statistical threshold and cluster size a pixel by pixel comparison of WMH was conducted using unpaired t-tests. In addition, all subjects underwent comprehensive neuropsychological testing.
Results: Depressed and control subjects did not differ in mean age 68.7 +/- 7.7 vs 68.5 +/- 6.2, years of education (14.2 vs 15.1) or gender (68% vs 66% female). WMH burden was greater in depressed than control subjects in four prefrontal deep white matter regions 1) L gyrus frontalis medialis (GFM) (BA 10) 91.8 mm3 vs 32.5 mm3 (p = .01), 2) R GFM (BA 46) 161.4 mm3 vs 61.6 mm3 (p = .01) 3) R GFM

and parts of gyrus frontalis superioris (BA 8) 31.0 mm3 vs 11.4 mm3 (p = .005) and 4) R cingulate gyrus (BA 8) 45.5 mm3 vs 17.7 mm3 (p = .01). Lesion burden in ROI 1 was correlated with processing speed r= -.25 (p = .01), episodic memory r = - .19 (p = .04) and executive function r = -.28 (p = .003). ROI 2 was correlated with processing speed r = -.22 (p = .02), episodic memory r = -.21 (p = .03), and executive function r = -.22 (p = .03). ROI 3 was correlated with working memory r = -.20 (p = .04). ROI 4 was correlated with executive function r = -.24 (p = .01).
Discussion: Fully automated objective segmentation of tissue types was achieved for all subjects in this sample. LLD subjects differed from controls in WMH volume in specific prefrontal white matter regions. WMH lesion burden in these regions was correlated with performance on tests of processing speed, executive function, working memory and episodic memory, suggesting that specific WMH lesions may be implicated in the cognitive impairment of LLD. Supported in part by MH60697 and K24-65421.

45. Effects of Lithium Treatment on Human Brain Structure Mapped Using Longitudinal MRI

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Background: Prior neuroimaging studies have shown cortical gray matter increases in lithium-treated bipolar patients, which may be related to treatment, rather than the disease process.
Methods: We studied serial high-resolution structural MRI brain scans (3D SPGR, 1.5 mm slices) from 13 healthy adult volunteers (6F/7M); mean age: 25.5 y ± 10.0 SD, scan interval: 4 weeks) treated with therapeutic doses of lithium (0.5-1.5 mEq/L). To map 3D patterns of local brain tissue changes over time, we used a novel intensity-based algorithm for non-linear elastic image registration in order to deform the follow-up to the baseline scan. A tissue change map (the Jacobian of the deformation), for each individual, was nonlinearly normalized to the anatomical space of a reference subject. Effects of lithium treatment were established by voxel-wise averaging the log-transformed Jacobian maps.
Results: These analyses revealed significant brain tissue increases following 4 weeks of lithium treatment, with a 2-4% average volume increase, most robustly in the right hemisphere, in parietal, primary sensorimotor and superior frontal regions. This pattern is consistent with tissue gain localized in prior cortical mappging studies of lithium-treated bipolar patients. Permutation testing on the 13 logged Jacobian maps, conducted voxelwise, indicated a p-value of 0.039 after multiple comparisons correction.
Discussion: Brain structural changes are detectable in healthy subjects treated with therapeutic doses of lithium over 1 month. These MRI changes may reflect postulated neurotrophic/neuroprotective effects of lithium, as prior studies have shown that lithium increases levels of the cytoprotective factors in the frontal cortex.

46. Marked Deficits in Membrane Phospholipid Precursor Levels in Attention-Deficit/Hyperactivity Disorder (ADHD) Children with Comorbid Diagnoses

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Sponsor: Matcheri Keshavan

Background: Attention-deficit/hyperactivity disorder (ADHD), is one of the most prevalent neurodevelopmental behavioral disorders. ADHD is first diagnosed in children with symptoms of inattention, hyperactivity and impulsivity. In a preliminary *in vivo* phosphorus (^{31}P) spectroscopy study [a noninvasive technique that can directly assess the metabolism of membrane phospholipids (MPL) and high-energy phosphates in multiple brain regions], we have shown deficits in MPL precursor levels in the basal ganglia (BG) and prefrontal (PF) regions of children with ADHD compared to healthy control subjects. The purpose of this study is to assess the effect of comorbid oppositional defiant disorder (ODD) and/or conduct disorder (CD) on *in vivo* MPL deficits. We hypothesized greater MPL deficits in ADHD subjects with greater severity in behavioral symptomatology.
Methods: Twenty-five children with DSM-IV ADHD [all males; mean age 8.8±1.5 yrs; 6.3-11.9 yrs; 16 stimulant-naive (nADHD) and 9 treated-ADHD (tADHD) subjects; 15 with the combined type and 10 with the predominantly inattentive type], and 22 healthy control subjects (HC; all males; mean age 9.2±2.1 yrs; 6.4-12.1 yrs) participated in this study. There were 13 ADHD subjects who had a comorbid ODD and/or CD diagnoses (coADHD). The MPL precursor levels [PME(s-τ_c)] and 5 other metabolites [MPL breakdown products PDE(s-τ_c), phosphocreatine, adenosine triphosphate , inorganic orthophosphate, and the broad peak underlying the phosphodiester resonance] were quantified in the PF and BG using a multi-voxel, *in vivo* ^{31}P spectroscopy technique. A generalized linear regression model (SAS Institute Inc., PROC GENMOD) with subject group, age and hemisphere as the main effect terms was used to test bilateral group differences in each region. A second model with an additional subject group-by-age interaction term was used (only two-group comparisons).
Results: The tADHD subjects were significantly older than the nADHD subjects; however, age was not significant between the ADHD subjects with or without comorbidity. **Total ADHD subjects vs HC:** The PME(s-τ_c) levels were significantly lower in the PF (p=0.0065) and BG (p=0.027) of ADHD subjects compared to HC subjects. There also was a significant age-by-group interaction (p=0.0021) with lower PF PME(s-τ_c) levels in the older ADHD subjects. **coADHD, ADHD and HC:** The PME(s-τ_c) levels were lower in the PF (p=0.0010) and BG (p=0.0009) of coADHD subjects compared to HC subjects, as well as being significantly lower in the BG of coADHD compared to the ADHD with no comorbidity (p=0.023).
Clinical correlations: Including all subjects, the ODD and externalizing t-scores (Child Behahavior Checklist-parent report) inversly correlated with the BG PME(s-τ_c) levels (r=-0.38 and p=0.0007; r=-0.40 and p=0.0003).
Discussion: Overall, there is a significant deficit in the MPL precursor levels in the PF of relatively older ADHD children and in the BG across the ADHD children suggesting decreased synthesis of MPL due to reduced membrane mass or content, which is consistent with an underdevelopment of neuronal processes and synapses in ADHD. Also, the marked reduction in BG MPL precursor levels in the coADHD subjects compared to both HC and the ADHD subgroup without any comorbidity along with the clinical correlations suggest greater MPL deficits with increasing ODD symptomatology and externalizing behavior in ADHD children. These MPL precursor deficits reflect a potential valuable marker of behavioral symptomatology among ADHD children.

47. GABRA2 Genetic Variation and Response of the Human Amygdala and Insula to Fearful Faces

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Background: The association of genetic variation in 5HTTLPR with amygdala response to fearful faces has been replicated several-fold. We have shown that amygdala (and insula) activation to emotional faces can be attenuated with a benzodiazepine (lorazepam) in a dose-dependent fashion. The anxiolytic effects of benzodiazepines are mediated through actions at the gamma-aminobutyric acid (GABA)-A

receptor. We tested the hypothesis that variation in the gene for the GABA-A receptor alpha-2 subunit (GABRA2, located on 4p12) is associated with amygdala and insula responsivity to fearful faces.
Methods: 21 healthy subjects 18-21 years old without DSM-IV Axis I disorders were recruited from a large pool of college students. Subjects participated in blood-oxygen level dependent functional magnetic resonance imaging (BOLD-fMRI) during an emotion face assessment task that has been shown to reliably engage the amygdala and associated limbic structures. Variation in GABRA2 was indexed using a non-coding SNP (rs279858) in strong linkage disequilibrium with other SNPs which, individually and as part of a haplotype block, have been associated with alcohol dependence. A linear regression analysis was performed with GABRA2 genotype (A/A, A/G, G/G) as the independent variable and % signal difference between fearful faces and ovals as the dependent measure. An a-priori region of interest analysis was performed with bilateral amygdala and insula as target areas.
Results: Individuals homozygous for the G allele had significantly greater activation in left amygdala relative to heterozygotes or A homozygotes. In addition, the G allele was associated with significantly greater activation in bilateral anterior insular cortex.
Discussion: This study adds to accumulating evidence of genetic influences on amygdala activation during emotion processing. In this study, variation in GABRA2 was associated with differences in the magnitude of amygdala and insula activation when processing fearful faces. Anxiety-prone individuals exhibit increased activity in amygdala and insula during performance of this task. Given the role of the GABA-A receptors as the targets of several important medications, notably anxiolytics, the relationship between individual drug response and differences in GABRA2-influenced variations in limbic responsivity should be further evaluated.

48. Diffusion Tensor Imaging: Fractional Anisotropy in White Matter, Symptom Severity, and Cognition in the Brains of Schizophrenia Patients
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Sponsor: Emmanuel Landau

Background: Data from a variety of sources and investigatory methods continue to garner support for the involvement of white matter in schizophrenia. Diffusion tenor imaging (DTI) provides a measure of the organization of tissue-fractional anisotropy (FA)-and DTI has been particularly informative about alterations in white matter coherence and organization in the brains of patients with schizophrenia. Our current study aimed at studying the relationship between regional fractional anisotropy and both symptom severity and cognition in schizophrenia patients.
Methods: Diffusion tensor imaging was performed on 63 schizophrenic subjects and 55 normal controls. Symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS), and cognition was assessed with an extensive neuropsychological battery from which a composite, standardized cognitive score was derived. The relationship between fractional anisotropy in each ROI and symptom severity and cognition were analyzed using Pearson Correlations. Forward stepwise regression analysis was performed in order to determine the contribution of each white matter ROI to the severity of symptoms or to cognitive performance.
Results: Significant correlations were found between left and right medial temporal white matter fractional anisotropy (L and RMT WM FA) and both positive and negative symptom severity. Fractional anisotropy in LMT WM inversely correlated with the severity of positive, negative, and general psychopathology. RMT WM FA inversely correlated with positive and negative symptom severity. Left dorsolateral prefrontal white matter fractional anisotropy (DLPF WM FA) inversely correlated with both positive and general symptom domains. Forward stepwise regression demonstrated that LMT WM FA accounted for a significant amount of the total variance in all three symptom domains. Only FA of WM in the left DLPF region correlated with the composite cognitive variable.
Discussion: Both positive and negative symptom severity may be partially determined by the organization of white matter tracts in schizophrenia as revealed by diffusion tensor imaging, in particular, by LMT and left DLPF WM tracts. Although RMT WM was significantly correlated with positive symptoms, the contribution of FA in this region did not add significantly to the total variance in symptom severity once the contribution of LMT WM was taken into account. Exactly how the organization of these WM tracts may impact symptom severity and cognition is not clear; however, anisotropy measures may have predictive and even prognostic value in schizophrenia.

49. Physiological Correlates of a Non-Working Memory Prefrontal Task
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Sponsor: James Dingell

Background: Dorsolateral prefrontal cortex has most often been studied with tasks that involve a working memory (WM) component. In an effort to characterize more fully frontal dysfunction in schizophrenia, we have examined blood-oxygen-level dependent (BOLD) activation in an event-related Digit Symbol Task, wherein subjects engaged executive processes without requirement for WM maintenance.
Methods: We studied 17 normal volunteers at 3T-fMRI. Each task trial required subjects to decide whether a number-symbol pair matched that in a corresponding numbered array of 9 unique number-symbol pairs appearing above the cue pair. There were equal numbers of non-matching and matching trials. Images were thresholded at $p<0.05$ corrected for false-discovery rate.
Results: For both the matching>rest and non-matching>rest trials, we observed areas of activation in bilateral dorsolateral prefrontal cortex (BA 9, 10, 46), inferior parietal lobule (BA 40) and thalamus. No significant activation was observed in the ventrolateral prefrontal cortex (BA 44, 45, 47).
Discussion: The Digit Symbol Task preferentially engaged dorsolateral prefrontal cortex in regions often associated with WM tasks. These results suggest that we might be able to parse those prefrontal regions involved in WM from those involved in other executive cognitive sub-processes. In the future, we will use this paradigm to study prefrontal dysfunction in schizophrenia.

50. The Development of Human Parent-Infant Attachment: Functional Brain Imaging During the Early Postpartum
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Sponsor: Past Travel Awardee, NIMH, 2003

Background: Parenting, a key foundational component of secure attachment critical to health, is many things from cultural creation to a psychological and biological transformation. Parenting includes a conserved set of thoughts and behaviors, some of which involve bear a resemblance to obsessions and compulsions. With the birth of a child, there may be preoccupations and anxious intrusive thoughts regarding the infant, along with a compulsive need to check the infant repeatedly until things are "just right". Recent studies in human mothers have shown that brain regions of stress response and salience

determination are activated by infant cries and picture stimuli. We are engaged in ongoing studies of parenting circuits longitudinally in the postpartum in both parents - when the intense emotions involved in parenting may be most intense. Specifically, hypothesize that: 1) baby cries activate cortical-subcortical circuits which correlate with levels of parental preoccupations involving anxious, intrusive, obsessive-compulsive-like thoughts; 2) baby pictures activate circuits of sensory analysis and reward, and correlate with preoccupations involving rewarding idealization and psychometric assessments of bonding to the baby; 3) brain activations and salivary cortisol responses to infant stimuli will be greater: for their own child, at the earlier postpartum time point, in moms versus dads, and in first-time versus veteran parents.
Methods: We are studying parental attachment in several ways in 30+ sets of parents: we are administering interview and self-report inventories of parental thoughts and actions, making videos of parent-infant interaction to assess attachment, performing functional magnetic resonance imaging (fMRI) of the brains of both mothers and fathers (using a Siemens 3T Trio scanner) as they attend audiovisual baby stimuli, and assaying salivary cortisol and urinary catecholamines with infant stimuli exposure. Also, all data are acquired longitudinally at 2 weeks and 3 months postpartum.
Results: fMRI brain activation maps from 2-4 weeks postpartum, comparing responses to own versus other baby cry stimuli, involve limbic and brainstem subcortical (alarm and attention) areas plus sensory and cingulate cortex (decision circuits). First-time parents activate more alarm centers than veteran parents, and mothers activate more than fathers. Over the first few months, alarm responses shift to hypothalamus (metabolic control), nucleus accumbens (reward), and frontal cortical (planning) activations as the parent-infant bond develops. Own baby pictures activate fusiform (face response) and insular (mirror/empathy) cortex more in moms than dads and depending on the time spent with the baby. Psychometric data indicate significantly higher preoccupations in moms compared to dads ($p<0,001$), and correlations of pre-occupations with intrusive worries and checking ($p<0.01$), depression ($p<0.001$), and brain activity in the amygdala and basal ganglia (fear, worry and OCD circuits) at 2 weeks; correlations hold at 3-4 months.
Discussion: This is the first longitudinal study to combine fMRI brain imaging in both mothers and fathers, with concurrent psychometric and endocrine measures, and using both audio and visual stimuli. Parental brain activations include circuits required for emotion, drive, salience, and habit - consistent with animal work on parental behaviors. Brain responses vary with gender, postpartum timing, type of stimuli, and quality of relationship. Further research on families with mental health vulnerabilities, as well as conditions such as postpartum depression and substance abuse, may yield biological models for protective and vulnerability factors in human family attachments.

51. Rolling with the Punches: Emotional Resilience and Ventral Prefrontal Cortex

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Background: Personality theorists have identified a set of attitudes and beliefs critical for coping effectively with adversity and change, termed emotional resilience. Laboratory measures of resilience, assessed through subjective reports, are highly reliable predictors of recovery from real-life adversity. Physiologically, individuals scoring high on resilience scales exhibit faster cardiovascular recovery when an anticipated threat does not occur. Neural substrates underlying this capacity to adapt to change and stress have significant import for psychiatric disorders, but the neural mechanisms of resilience remain largely unclear.
Methods: This study used functional magnetic resonance imaging (fMRI) to identify neural substrates of resilience during recovery from negative expectations. Thirty healthy individuals were selected based on scoring in either the upper (high resilient, n=15, mean age=20.2 yrs, 5F, 10M) or lower (low resilient, n=15, mean age=20.5 yrs, 5F, 10M) quartiles on a resilience scale (ER89). To induce negative expectations, participants were first given one of two cues: the safety cue indicated that a neutral picture would appear (100 % probability); the threat cue indicated that either a neutral or an aversive picture would appear (50/50 ratio). Pictures appeared for 3 sec, 4-12 sec after the cue. BOLD-sensitive fMRI scans were obtained using a reverse spiral sequence while subjects passively viewed the cues and pictures, with jittered intervals (4 - 12 sec) to permit deconvolution of signal from each epoch of the experimental trial. Realigned and normalized images were analyzed in a standard, random effects model.
Results: Both expectations for and experience of aversive events (pictures) elicited activity in the right ventrolateral prefrontal cortex (VLPFC). When negative expectations were followed by better-than-expected events, activity in resilient individuals was suppressed in the right VLPFC, suggesting negative expectations were rapidly updated by new experience. In contrast, non-resilient individuals showed sustained activity of the VLPFC, regardless of the stimulus that followed the threat cue (50, 31, -12; Z=3.74; 47, 22, 6; Z=3.27). Low resilient individuals also showed greater activation of ventromedial prefrontal cortex (VMPFC: -3, 50, -15; Z=5.52) during the threat cue, when they anticipated an aversive picture, in the same region activated by both groups when an aversive picture did appear (0, 50, -19; Z=4.85).
Discussion: This preliminary study suggests that prefrontal networks involved in the regulation of emotion — VLPFC,VMPFC — differentially regulate the expectation and experience of an aversive event as a function of an individual's psychological resilience. The VLPFC has been implicated in inhibitory control and emotion regulation, and the present findings suggest that high resilient individuals are able to 'switch off' this region as a part of their faster recovery from the expectation of a negative event. How these regions interact with other brain regions to deal with adversity and change should have important implications for understanding individual differences in the susceptibility to psychiatric illness.

52. Evidence for Sensitive Periods in the Effects of Childhood Abuse on Regional Brain Morphology

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Sponsor: Susan Andersen

Background: The brain is molded by experiences that occur throughout the lifespan. However, there are particular stages of development when experience exerts either a maximal (sensitive period) or essential (critical period) effect. For example, Hubel and Wiesel found that binocular deprivation maximally affected development of the visual cortex in cats if it occurred early in postnatal life, but had no impact after puberty. Little direct evidence exists for sensitive or critical periods in human brain development. Based on differential rates of maturation specific brain regions should have their own unique periods of sensitivity to the effects of early experiences such as stress.
Methods: To ascertain if this is true in humans, the size of apriori selected target regions were measured from high-resolution volumetric MRI scans (1.5 T GE Echospeed) from 26 unmedicated right-handed collegiate females (18 -22 years old) with a history of repeated childhood sexual abuse and 19 healthy sociodemographically comparable controls. Hippocampus and amygdala were manually traced in their entirety according to the method detailed by Pruessner et al (2000). Anatomical measurements of corpus callosum area were obtained from the midsagittal image. An automated algorithm created in NIH Image divided the manually-traced corpus callosum into seven regions as defined by Witelson (1989). Grey matter volume (GMV) of the prefrontal cortex was assessed using optimized voxel-based morphometry (VBM) computed using SPM2 in MatLab 6.5. Results were

verified using a semiautomated program for cortical surface-based analysis (FreeSurfer).
Results: Based on stepwise regression hippocampal volume was particularly sensitive to abuse that occurred at 4 years of age ($p<0.001$). In contrast, the midsaggital area of the rostral body of the corpus callosum was affected by abuse that occurred at age 9 ($p<0.05$), while grey matter volume of the prefrontal cortex was affected by abuse at age 14 ($p<0.02$).
Discussion: Early episodes of repeated childhood sexual abuse were associated with alterations in regional brain size. However, within the same group of subjects it appeared that there were marked differences between regions in the ages of greatest vulnerability. These findings provide the first evidence in humans that brain regions with different rates of maturation have unique windows of vulnerability to the effects of early traumatic stress.

53. Allelic Variation of PPP1R1B, Encoding DARPP-32, Affects Human Striatal Volume and Striatal-Prefrontal Structural Connectivity
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Sponsor: Samuel Kaim

Background: Dopamine and cyclic AMP-regulated phosphoprotein of relative molecular mass 32,000 (DARPP-32) is a central regulator of dopaminergic and glutamatergic signal transduction. It can act as an inhibitor of both protein phosphatase-1 (PP1) and protein kinase A and, as such, is in a unique position to mediate phosphorylation and dephosphorylation of signal transduction across many pathways in the brain. By far the highest expression of DARPP-32 is found in medium spiny neurons of the striatum. Functional interactions of the striatum and prefrontal cortex (PFC) play an important role in executive function and reward, are critically modulated by dopamine, and are disturbed in schizophrenia, depression, and drug abuse. In healthy subjects, voxel-based morphometry (VBM) has been useful in identifying alterations in gray matter volume related to allelic variation in genes (e.g. BDNF, SERT, and DISC1). To date, no studies have investigated the effect of DARPP-32 genetic variation within a neuroimaging framework. The current study utilizes VBM to examine the impact of the DARPP-32 haplotype on gray matter volume in the human brain. Given its known function in regulating signaling in the striatum, we hypothesized that DARPP-32 genetic variation would impact striatal volume. In addition, structural covariance analysis was used to investigate correlations in volume between the striatum and prefrontal cortex as a measure of "wiring" of that circuit.
Methods: We studied 96 healthy volunteers using VBM (47 male; 49 females; age range 19-60; all Caucasian of European ancestry with no current or prior history of psychiatric or neurological illness). T1-weighted SPGR MRIs were acquired on a 1.5T GE scanner with a voxel resolution of 0.975 x 0.975 x 1.5 mm3. For data processing, VBM was performed in SPM2 using an optimized VBM protocol with customized apriori templates. Data were analyzed using the General Linear Model, as implemented in SPM2, with linear and nonlinear age expansions, as well as sex and total gray matter volume, used as covariates. We analyzed a noncoding SNP (rs879606) tagging a haplotype block spanning the gene, comparing subjects homozygous for the frequent allele to carriers of the rare allele. Averaged seed voxels in left and right putamen were used for structural covariance analyses.
Results: We found significant gray matter volume differences between DARPP-32 genotypes in the striatum; individuals homozygous for the frequent allele had decreased gray matter volume, particularly in the left putamen. Genotype was associated with striking differences in structural interactions between striatum and prefrontal cortex: Subjects homozygous for the frequent allele showed strongly increased connectivity from putamen to bilateral dorsolateral prefrontal cortex, while striatal connectivity with supplementary motor cortex was decreased.
Discussion: DARPP-32 functions at the "crossroads" of several neurotransmitter systems, acting as an integrator of cell signaling. Our analysis reveals genotype effects on striatal volume and structural interactions with prefrontal cortex that might underlie changes in fronto-striatal information processing and contribute to risk for psychiatric disease, especially schizophrenia. Further investigation using genetic association, cognitive testing and functional neuroimaging, will help more fully elucidate the neurobiological consequences of DARPP-32 genetic variation.

54. Gastric Stimulation Activates Brain Satiety Circuiy
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Sponsor: Nora Volkow

Background: Gastric stimulation is a new obesity treatment that leads to decreased food intake and reduced body weight. The neurobiological mechanism underlying the decreased food intake from gastric stimulation is unknown. Here we use the Transcend® Implantable Gastric Stimulator (IGS®) system, which generates electric signals to induce the expansion of the fundus, to assess the effect of gastric stimulation on regional brain activity in obese subjects.
Methods: Three female and 1 male obese subjects (49.1 ± 5.8 yrs of age) who had the IGS implanted for 1-2 years and had maintained positive therapeutic response (excess body weight loss of $29 \pm 12\%$) were evaluated with PET and FDG. Brain metabolism was evaluated in food deprived (17-19 hours) subjects during activation (on) and deactivation (off) of the IGS in separate days (> 2 weeks). The absolute metabolic images were analyzed using Statistical Parametric Mapping. The Three Factor Eating Questionnaire-Eating Inventory (TFEQ) was obtained on the day of scans to measure restraint, disinhibition and hunger components.
Results: The subjects had significantly higher scores in hunger during deactivation (8.5 ± 3) than activation (6.38 ± 3.2, $p < 0.05$). Comparison of the metabolic images show that during activation subjects had significantly higher ($p < 0.005$) activity in the left thalamus, left mesencephalon, left pons and cerebellar vermis ($p < 0.005$) than during deactivation.
Discussion: Activation of the mesencephalon, the pons, and the the thalamus which are regions that contain nuclei involved in the regulation of satiety and motivation to eat respectively suggest that this is the mechanism by which gastric stimulation decreases food intake. It also suggest that the vermis a region that is not typically associated with appetitive behaviors may also be involved with food intake. Supported by DOE (OBER) and NIH/NCRR (5-MO1-RR-10710).

55. 123I-ADAM SPECT Study in Patients with Major Depression: Serotonin Transporter Occupancy Induced by Paroxetine and Relationship with Clinical Response
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Sponsor: Past Travel Awardee, ECNP-ACNP Fellow, 2003

Background: The aim of this study was to investigate the degree of SERT occupancy (%SERTocc) induced by paroxetine in patients with major depression disorder (MDD) by means of 123I-ADAM SPECT, and explore the possible relationship with the clinical response, reduction in the Hamilton score after 6 weeks of treatment.
Methods: Ten patients (6 F, 36,12.0 yrs) with DSM-IV criteria of MDD underwent two SPECT scans, one at baseline (before treat-

ment) and another between 4-6 weeks (38.9, 6.4 days) of treatment with paroxetine 20 mg/day. Hamilton-D (HAMD) scores were recorded at baseline and after treatment, and a decrease of >50% from the baseline score was considered as clinical response. Image acquisition started 4h. after i.v. injection of 123I-ADAM (167.7,63.8 MBq). MRI-SPECT coregistration was performed and used for ROI drawing and placement on the midbrain (including raphe nuclei) and cerebellum (C). Specific uptake ratios (SUR) were obtained as [(midbrain-C)/C]. %SERTocc was calculated as 100x [(SURbaseline-SURparoxetine)/SUR baseline].
Results: Mean HAMD scores were 20.9,2.7 at baseline and 9.6, 2.9 after treatment. MDD patients showed a mean of 53.8, 12.9% improvement in HAMD and a mean %SERTocc of 64.1, 9.8% (range 36.25-77.7%). One patiente dropout the study before the second SPECT, two patients did not show clinical response, less than 50% change in HAMD from baseline. One of these patients showed a %SERTocc >60%. The Correlation coefficient (r) between %RO and the Hamilton reduction was 0,71 ($p < 0.05$)
Discussion: These preliminary results suggest that antidepressant treatment with paroxetine 20 mg/day leads to a mean %SERTocc greater than 60%, and that this degree of %SERTocc is associated with clinical response to treatment. 123I-ADAM SPECT seems to be appropriate for the assessment of drug-induced %SERTocc in MDD patients. Marato TV3 exp. 011410

56. Epistasis of SERT & BDNF: A Mechanistic Model of Depression
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Sponsor: Daniel Weinberger

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

57. Associations in the Longitudinal Course of Body Dysmorphic Disorder with Major Depression, OCD, and Social Phobia
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Background: Body dysmorphic disorder (BDD), a distressing or impairing preoccupation with an imagined or slight defect in one's physical appearance, is a relatively common and severe somatoform disorder that is highly comorbid with certain other Axis I disorders. BDD is widely conceptualized as an OCD-spectrum disorder, based on its prominent obsessions and compulsions, high comorbidity with OCD, apparent preferential response to SRIs, and increased frequency in first-degree relatives of OCD probands compared to community controls. BDD has also been hypothesized to be related to mood disorders, given its high comorbidity with major depression and response to antidepressants (SRIs). In Eastern cultures, in contrast, BDD is considered a form of social phobia. However, differences have been found between BDD and these frequently comorbid disorders, suggesting that BDD is not identical to any of them. The relationship between BDD and these putative "near neighbor" disorders has received little investigation, however. To investigate these relationships, which have treatment implications, we examined longitudinal associations between the course of BDD and that of comorbid major depression, OCD, and social phobia in the first prospective study of BDD's course.
Methods: 161 subjects with DSM-IV BDD were prospectively followed over 1 to 3 years. The course of BDD and comorbid disorders was assessed with the reliable Psychiatric Status Rating Scale (PSR); ratings were assigned for each week of follow-up. To examine the longitudinal association between improvement in BDD and improvement in each comorbid disorder, we used proportional hazard regression analyses with time-varying covariates. This approach enables examination, among subjects with both disorders in a given pair at baseline (e.g., both BDD and major depression), of whether changes in course are correlated. If they are, that strongly suggests they are etiologically linked to each other or to a third factor leading to the correlated change. In the prediction of remission of a particular disorder, the time-varying predictor was the PSR rating for the comorbid disorder during the week preceding the time point being analyzed. Hazard (risk) ratios provided an estimate of strength of association between the predictor and dependent variables.
Results: BDD had significant longitudinal associations with major depression—that is, change in the status of BDD and major depression were closely linked in time. Improvement in major depression predicted remission from BDD (HR=.604, p=.0006), and, conversely, improvement in BDD predicted remission from major depression (HR=.745, p=.0028). Improvement in OCD also predicted remission from BDD (HR=.506, p=.005); however, BDD improvement did not predict OCD remission (HR=.878, p=.542). No significant longitudinal associations were found for BDD and social phobia. Among those

subjects who remitted from major depression, OCD, or social phobia, a majority continued to experience BDD symptoms over the following three months.
Discussion: These findings suggest that BDD may be etiologically linked to both major depression and OCD; the association was strongest for depression. However, BDD does not appear to simply be a symptom of these comorbid disorders, as BDD symptoms persisted in a majority of subjects who remitted from these disorders. This finding implies that BDD symptoms specifically (not just those of these commonly comorbid disorders) need to be targeted in treatment. Additional research, including neurobiological research, is needed to further elucidate BDD's relationship to co-occurring disorders, which has both theoretical and clinical implications.

58. Toward the Identification of Biological Markers of Bipolar Disorder: A First Stage Neuroimaging Study
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Sponsor: Past Travel Awardee, Memorial, 2004

Background: Bipolar disorder (BP) is a debilitating and common illness. While depression is the most common presentation, in those without a clear history of mania this is often misdiagnosed as unipolar depression. Increased diagnostic accuracy of BP depression is therefore a key goal to improve the mental health of those with BP. This can be facilitated by the identification of measures reflecting pathophysiologic processes underlying core clinical features of the disorder, notably, impaired emotion regulation and attention. As a first stage toward the above, we aimed to measure functional neural abnormalities persisting through depression and remission during cognitive and affective processing in individuals with the traditional BPI subtype. Findings from the few existing studies examining functional neural abnormalities in BPI during task performance allowed us to hypothesize that: 1. Remitted BPI (RBPI) compared with healthy individuals (H) would show increased amygdala and decreased dorsolateral prefrontal cortical (DLPFC) activity to emotive stimuli; and decreased DLPFC activity during attention task performance 2. DBPI would show a similar pattern of abnormal neural response as RBPI overall, but relatively greater DLPFC activity than RBPI during attention task performance.
Methods: Using fMRI we measured in RBPI and DBPI compared with H neural responses during: A. emotion processing (gender labeling of mild and intense happy and fearful facial expressions); B. attention and working memory (digit sorting, in which participants remember the middle digit of either 3, 4 or 5 digits); and C. regulation of emotion during attention (digit sorting after positive and negative emotion word labeling).
Results: Facial expression labeling: our findings to date indicate for mild happy expressions a significant interaction between scan time x group (BPI vs. healthy; mixed model random effects ANOVA: $p<0.01$). All BPI showed early increased right amygdala activity, while DBPI showed increased and more sustained amygdala activity to these happy expressions than other groups. All BPI also showed decreased left DLPFC activity (Brodmann Area, BA, 46) $p<.005$. To intense fear expressions, all BPI showed less sustained activity compared with H in left DLPFC (BA9). Digit sorting: there was a significant interaction between task condition (number of digits) x scan time: random effects repeated measures ANOVA: $p<.005$: all individuals showed increased activity in right DLPFC (BA 9) and anterior cingulate gyrus (BA32) for 5 s 3 digit sorting. RBPI showed reduced, but DBPI increased, activity in these regions compared with H for 5 versus 3 digit sorting. Alternating Emotion Word Labeling and Digit Sorting: there was a significant interaction between group (all BPI versus healthy) x task condition (positive, negative, neutral word) x scan time: random effects repeated measures ANOVA: $p<.005$, in left amygdala and left DLPFC. RBPI showed more sustained left amygdala activity after negative words than H, while DBPI showed increased DLPFC (BA9/ 46) activity after positive words.
Discussion: These data support the presence of persistent functional abnormalities in neural systems mediating emotion regulation and attention in BPI. Our findings also suggest a positive emotion neural response bias in depressed individuals with BPI, distinct from the negative emotion response bias previously observed in depressed individuals with unipolar depression. These preliminary findings highlight the utility of neuroimaging techniques in the identification of potential biological markers of bipolar disorder.

59. Shared Genetic Contributions to Pathological Gambling and Major Depression in Men
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Sponsor: Marc Potenza

Background: Although pathological gambling (PG) and major depression (MD) frequently co-occur, little is known regarding the relative contributions of environmental and genetic factors to the co-development of the disorders. The objective of the present study was to estimate environmental and genetic contributions to DSM-III-R PG and MD and their lifetime co-occurrence.
Methods: Survey data from the Vietnam Era Twin Registry were examined in biometrical models. 7,869 of 10,253 eligible participants were successfully interviewed via telephone. Estimated genetic, shared environmental, and unique environmental contributions to PG and MD and their lifetime co-occurrence were estimated in bivariate models.
Results: Elevated odds ratios (ORs) for MD were associated with PG (4.06; 95% Confidence Interval (CI): 2.68-6.13), and the association remained significant following adjustment for sociodemographic and other psychiatric variables (OR = 1.98; 95%CI= 1.14-3.45). The best-fitting bivariate model indicated that 66% of the variance in PG and 41% of the variance in MD were due to genetic factors, and 34% of the variance in PG and 59% of the variance in MD were due to unique environmental factors. There was a substantial correlation between the genetic components of PG and MD ($rA=0.58$), with 34% of the genetic variance for each disorder also contributing to that for the other. The best-fitting model estimated 100% of the total overlap between PG and MD to be genetic.
Discussion: The correlation between PG and MD in middle-aged men appears to be largely influenced by overlapping genetic factors. Future research is needed to determine the extent to which these findings extend to other groups (e.g., women), identify specific genes, and generate improved prevention and treatment strategies.

60. Adolescent Depression and Neuronal Circuits Associated with Reward Processes
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Background: Adolescent depression is frequently associated with significant morbidity and mortality. The episodes often persist into adult life along with ongoing psychosocial difficulties. Given the socioeconomic burden associated with adolescent depression, a better understanding of the pathophysiology of this disorder will be helpful in developing effective interventions. In considering the pathophysiological processes associated with adolescent depression, responses to reward are of interest because of the presence of symptoms reflecting abnormal experience of pleasure in this disorder. Alterations in the experience of pleasure and motivation can be conceptualized as abnormal sensitivity and response to reward, indicating dysfunction in reward-related circuits. Such dysfunction can affect decision-making,

expectations of positive outcomes, and response to reward. Both clinical and preclinical data suggest that neuronal circuits involved in motivational processing continue to develop until late adolescence, and it is postulated that the dramatic changes in these circuits during adolescent development might place vulnerable youth at risk for depression. However, until recently, neuropsychological and neuroimaging studies of reward-related symptoms in depression have included only adults. The current study was undertaken to examine the neuronal substrates of reward processes in adolescent depression.
Methods: Twenty-two adolescents with major depressive disorder and 23 adolescents with no personal or family history of psychiatric illness were recruited. Using an event related design, fMRI images with BOLD contrast were collected during a two-choice probabilistic reward task with varying levels of risk. Behavioral performance also was recorded during the session. In each trial, subjects were asked to select one of two options based on the likelihood and magnitude of a gain. Two active conditions were used: a 25/75 wheel (25% chance of winning $6/$3, or 75% chance of winning $2/$1), and a 50/50 wheel (with equal chances of winning a given dollar amount). A plain wheel condition (with no dollar amount) was included as a control condition. A General Electric 1.5 Tesla scanner was employed. Gradient echo planar images were acquired in 26, 5 mm Sagital slices per brain volume. Two sets of analyses were conducted: (1) modulation of activation by reward/risk, high reward/risk events vs. the low reward/risk events; and (2) all monetary conditions (25/75, 50/50) vs. the control condition. In addition to this voxel-based analysis, Region of Interest (ROI) analysis was performed.
Results: The groups did not differ significantly with respect to age, gender or behavioral performance on the task. In the fMRI contrasts of monetary reward selection vs. control condition, normal volunteers activated anterior cingulate, ventral striatum and ventromedial prefrontal cortex while the depressed subjects did not activate these neuronal circuits. In the high reward/risk vs. low reward/risk contrast, controls activated anterior cingulate and ventral striatum whereas depressed subjects failed to activate these regions.
Discussion: The observed brain activation patterns in normal controls are particularly relevant for processing the various stages of decision-making, and include structures such as orbitofrontal cortex, anterior cingulate, ventral striatum, dorsolateral prefrontal cortex and parietal cortex. Consistent with our findings, structural and functional neuroimaging studies in adult depressed patients showed dysfunction within the prefrontal cortical and striatal systems that normally modulate limbic and brainstem structures involved in mediating emotional behavior in the pathogenesis of depressive symptoms.

61. Depression-Insulin Resistance Link

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Sponsor: Uriel Halbreich

Background: Elevated androgen levels have been associated with depression in women, and women with hyperandrogenic syndromes are at a considerable risk for mood disorders. Further, increased androgen levels are associated with both insulin resistance (IR) and obesity. Insulin is known to increase ovarian androgen production in women with polycystic ovary syndrome (PCOS); while conversely, androgens can cause IR in women. We previously reported a relationship between mood and IR, as we found increased rates of depression in women with PCOS. Hence we postulated that IR may link mood and endocrine disorders.
Methods: We present data from our and others studies on the relationship between IR and mood in various clinical populations.
Results: 1) Prevalence of IR in patients with bipolar disorder In both cross-sectional and longitudinal studies of reproductive function in women with bipolar disorder (BD), we found that 57% and 80 % of subjects, respectively, exhibited IR, measured with the homeostasis model assessment (HOMA) score>2.3, at baseline. In the longitudinal study, 85% of subjects exhibited IR at two year follow-up. 2) The prevalence of bipolar spectrum symptoms in patients with PCOS; The prevalence of PCOS in the general population of reproductive-aged women has been estimated to be between 4% and 7%, although it could be as high as 11%. Klipstein and Goldberg recently found that 28% of subjects diagnosed with PCOS also met Mood Disorders Questionnaire criteria threshold criteria or had a previous diagnosis for a bipolar spectrum illness. 3) The prevalence of IR in patients with unipolar depression In 20 patients with untreated unipolar depression, mean fasting plasma insulin levels were 27.42 and mean fasting plasma glucose levels were 86.26. Additionally, mean HOMA levels were 5.94 and average body mass index (BMI) was 26.69 for subjects assessed. 4) The relationship between depression and PCOS. In a study of 32 women diagnosed with PCOS, we found that 16 (50%) exhibited Center for Epidemiological Studies-Depression Scale (CES-D) scores of greater or equal to 16, indicating depression. Untreated depressed patients exhibited significantly higher BMI and IR values than untreated nondepressed patients. Depression was also associated with greater IR (P=0.02) and higher BMI (P=0.05).
Discussion: Women with unipolar depression and BD have higher HOMA ratios as well as women with primary IR syndrome (e.g. PCOS) have higher rates of depression. As a majority of women in all studies were at least overweight, obesity and weight gain may underlie high rates of IR in depressed populations. The interrelationships among IR, mood and BMI require further investigation, but presented findings suggest that IR may link disorders of mood and disorders of reproduction.

62. Pediatric Bipolar Disorder and Face Processing: Neural and Behavioral Deficits

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Sponsor: Travel Awardee, ADAA, 2005

Background: Aberrant emotion-attention interactions may be central to the emotional and behavioral dysregulation characteristic of pediatric bipolar disorder (PBD). For example, studies have shown that whereas PBD attentional functioning may be comparable to controls in non-emotional conditions, in emotional environments, children with PBD misallocate their attentional resources away from the primary task and become distracted by frustration, resulting in emotional and behavioral deficits. A related impairment characteristic of PBD is difficulty identifying emotional facial expressions, which may also reflect attentional deficits associated with emotional stimuli. Limbic neural regions, specifically the amygdala, striatum, and orbitofrontal cortex (OFC), have been implicated in emotion-attention interactions and face expression identification, and have also been implicated in the pathophysiology of PBD. However, neural activation in PBD in response to facial expressions has yet to be examined.
Methods: Participants were 21 healthy controls and 22 children with PBD (ages 8 to 17 years) equivalent in age, sex, and IQ. PBD subjects had strictly defined DSM-IV bipolar disorder, including a history of at least one episode (> 4 days) of hypo/mania including euphoric mood. Participants viewed neutral, happy, fearful, and angry faces while rating hostility and their own resulting fear (emotional ratings), as well as nose width (a non-emotional rating). Rapid event-related fMRI BOLD signal on a 3T scanner and behavioral data (ratings and reaction time) were collected.
Results: PBD subjects, compared to controls, rated neutral faces as more hostile and fear-producing. These ratings were associated with slower reaction times in PBD subjects. There were no behavioral differences during the non-emotional ratings of neutral faces. Consistent with behavioral results, fMRI contrasts found hyperactivation of the amygdala, striatum (accumbens and putamen), and OFC in PBD

subjects when conducting the emotional ratings of neutral faces, but there were no group differences in neural activation during non-emotional ratings. Differences in neural activation remained when controlling for between-group behavioral differences. Neural over-activation in the PBD sample did not differ based on mood state during testing, comorbid anxiety or ADHD diagnoses, or the use of psychotropic medications.
Discussion: Current results demonstrate the manner in which perturbations in emotion-attention interactions impair functioning in children with PBD. PBD children displayed behavioral and neurophysiological deficits when viewing neutral facial expressions, but only when subjects' attention was directed toward an emotional aspect of the face. Importantly, these negative interpretations of faces in PBD children were associated with increased activation of key limbic structures- the amygdala, striatum, and OFC. PBD neural deficits may reflect trait deficits given that limbic hyperactivation appeared to be independent of various demographic variables, including mood and medication status. Results also indicate that social deficits characteristic of PBD may be secondary to deficits in interpreting and responding to ambiguous or benign faces, which themselves may be associated with neural hyperactivation. Results implicate deficits in limbic brain regions in the pathophysiology of PBD.

63. Role of CRF2 Receptors in Maintenance and Recovery from CRF1 Receptor-Induced Alterations in Defensive Startle
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Sponsor: Travel Awardee, ADAA, 2005

Background: Patients suffering from panic disorder exhibit reduced acoustic startle habituation and deficits in sensorimotor gating measured as prepulse inhibition (PPI) of acoustic startle. Individuals with post-traumatic stress disorder also exhibit exaggerated startle responding and sensorimotor gating deficits. The pathophysiology underlying these disorders is hypothesized to include dysregulation of Corticotropin Releasing Factor (CRF). Studies in rodents demonstrate that exogenous CRF administration or endogenous CRF release increases startle responding and reduces PPI. CRF has two known receptors, CRF1 and CRF2. Recently we reported that CRF-induced deficits in PPI and increases in startle are reversed by CRF1 selective antagonism or in mice homozygous for CRF1 receptor gene deletion (CRF1 KO mice), indicating that activation of CRF1 reduces PPI while increasing startle. We also reported that the CRF2 antagonist antisauvagine-30 potentiates CRF-induced deficits in PPI while attenuating CRF-induced increases in startle, indicating that CRF2 activation increases PPI and may act in concert with CRF1 to increase startle. Based on these and other data we have developed a model of the respective roles of CRF receptors in defensive behavior during both acute and chronic CRF release. We suggest that CRF1 activation increasing defensive behaviors while CRF2 activation can both maintain and initiate recovery of CRF1-initiated stress responding.
Methods: To test our model, we examined the effects of h/r-CRF (2 μg, 5 μl/ICV) administration on acoustic startle magnitude and PPI in CRF2 KO mice over 5 hours.
Results: h/r-CRF administration significantly reduced PPI in wild-type (WT) mice. In CRF2 KO mice, however, h/r-CRF-induced disruption of PPI was significantly greater as compared to WT mice. Hence both pharmacological and genetic blockade of CRF2 functions appears to significantly exacerbate CRF-induced disruptions of sensorimotor gating. Intra-session startle habituation was also increased in CRF2 KO mice, indicating a possible role for CRF2 in modulating habituation of startle. CRF2 KO mice also exhibited a slightly longer time-course of recovery from CRF effects on startle.
Discussion: These data support the hypothesis that CRF2 receptors function to oppose CRF1 receptor-mediated effects on PPI, and hence may act to recover normal sensorimotor gating after CRF1-mediated disruptions. Additionally, the intriguing findings that CRF2 plays a role in blocking startle habituation yet reduces the time to recovery of normal startle responding after CRF administration supports further testing of the maintenance and recovery model of CRF2 effects on CRF1-mediated stress responding. Overall, the present findings provide further support for the heuristic value of our proposed model in which CRF1 and CRF2 receptors have additive influences on initial phases of stress-induced defensive responses and yet opposing influences on the subsequent recovery from or inhibition of these responses. These studies were supported by NIH grants DA02925, MH68133 and MH42228 as well as the Veteran's administration VISN 22 MIRECC. All studies were in accordance with the NIH guidelines for the care and use of laboratory animals and were approved by UCSD IACUC.

64. Safety and Tolerability of the Selegiline Transdermal System 20 mg for Treatment of Major Depression
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Background: The selegiline transdermal system (STS) is a monoamine oxidase (MAO) inhibitor with unique pharmacokinetic and pharmacodynamic properties. Through avoidance of both first pass metabolism and direct contact with gastrointestinal mucosa, STS provides concentrations of selegiline necessary for antidepressant activity while preserving intestinal MAO-A activity[1]. Previously reported controlled trials have demonstrated the effectiveness of STS 20 mg (per 20 cm^2)[2,3]. Herein, we review safety and tolerability data from short-term and long-term trials of patients treated with STS 20 mg. All trials, with the exception of an initial trial, were conducted without dietary tyramine restrictions.
Methods: Data from 1,326 patients with major depressive disorder (MDD) who received STS 20 mg were analyzed from available trials, as reported to the Food and Drug Administration. Placebo-controlled data were pooled for separate analyses (STS=534, placebo=535) to compare adverse event (AE) incidences. Computerized algorithms utilizing AE terms possibly indicative of untoward events associated with oral MAO inhibitors (e.g., acute hypertensive reactions, orthostatic symptoms, serotonin syndrome) were employed to search for these clinical events in the database.
Results: No tyramine-induced hypertensive episodes occurred in STS-treated patients, despite the lack of dietary tyramine restrictions. A computer search of the STS database yielded no evidence of clinical events that have been associated with oral MAO inhibitors (e.g., acute hypertension, severe orthostasis, serotonin syndrome). In controlled trials, 75% of STS-treated patients and 71% of placebo-treated patients experienced AEs, most of which were mild or moderate in severity. Two AEs with higher incidences compared with placebo were application site reactions (ASRs) (21.8% vs. 9.7%) and insomnia (8.6% vs. 4.9%). These side effects infrequently led to premature discontinuation for STS-treated patients: 3.5% for ASRs and 1% for insomnia in all trials. The incidence of sexual dysfunction, weight gain, gastrointestinal disturbances and orthostatic hypotension AEs was low and similar to placebo.
Discussion: Clinical experience in patients with major depression treated with transdermal selegiline 20 mg without dietary restrictions indicates that STS is a safe and well tolerated MAO inhibitor antidepressant. [1]Mawhinney M, et al. *J Pharm Pharmacol* 2003;**55:**27-34. [2]Bodkin JA, et al. *Am J Psychiatry* 2002;**159:**1869-1875. [3]Amsterdam JD, et al. *J Clin Psychiatry* 2003;**64:**208-214.

65. Alterations in Human Amygdala Corticotropin-Releasing Factor (CRF) Receptor Binding Associated with Psychiatric Illness
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Sponsor: Ned Kalin

Background: Psychological stress participates in the etiology or maintenance of psychiatric illness. The corticotropin-releasing factor

(CRF) system and the amygdala regulate many aspects of how the body responds to stress, and alterations in the brain CRF system and amygdala activity have been associated with depression and anxiety disorders. The goal of the present study was to describe the distribution of the two known CRF receptors (CRF_1 and CRF_2) in the human amygdala and associated temporal cortical tissue obtained postmortem, and to see if CRF receptor levels were altered in psychiatric illness.
Methods: *In vitro* autoradiography with [^{125}I]suavagine was used to assess CRF receptor levels in amygdala (basal, lateral, and ventral basolateral nucleus (vBLA)) and cortical (entorhinal and fusiform gyrus) regions.
Results: In normal subjects (N=11-15), CRF_1 binding was highest in the vBLA (503±54 pCi/mg). In addition, CRF_1 binding was higher than CRF_2 in all regions except the entorhinal cortex. CRF_2 binding showed a unique distribution with highest levels in the entorhinal cortex (1054±148 pCi/mg), and dramatically lower levels in the adjacent fusiform gyrus (51±15 pCi/mg). There were no differences in CRF receptor binding in tissue obtained from patients with schizophrenia, bipolar disorder or major depression compared to normals (N=9-15/group). Importantly, CRF_1 binding was lower in the vBLA of suicides pooled from all diagnostic groups (333±58 pCi/mg) compared to normals (503±54 pCi/mg; N=12/group), with a trend for a decrease in the lateral amygdala. In the same analysis, there were no changes in CRF_2 binding.
Discussion: This is the first characterization of CRF receptor binding in the human amygdala and associated cortex, and the first demonstration of changes in human amygdala CRF receptor binding associated with psychiatric illness. Support contributed by The Stanley Foundation, NIH grants MH40855 (NHK), MH65109 (RJH) and the University of Wisconsin HealthEmotions Research Institute.

66. A "Pharmacological Stressor" Model of Anxiolysis in Monkeys: Alprazolam Attenuation of the Behavioral and Physiological Effects of Yohimbine

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Background: The understanding of mechanisms underlying the action of anxiolytic compounds may be facilitated by the development of improved behavioral and physiological models using nonhuman primates. One potential approach is based on the use of a "pharmacological stressor", a compound that repeatedly induces stress responses in both human and non-human primates. The α2-adrenoceptor antagonist yohimbine has been shown to increase behavioral and physiological markers of stress in squirrel monkeys as well as subjective ratings of anxiety in humans. We explored the extent to which yohimbine-induced markers of stress in monkeys can be attenuated by a known anxiolytic compound (the benzodiazepine alprazolam).
Methods: Adult male squirrel monkeys were administered yohimbine (0.03-0.56 mg/kg, i.m.) alone or combined with alprazolam (0.1-1.0 mg/kg, i.m.) or saline prior to a videotaped session in an observation chamber. Following the session, salivary cortisol was determined in awake, unrestrained monkeys as described previously (Am J Primatol 60: 69-75, 2003). Videotapes were scored for the occurrence of self-directed behaviors (grooming and scratching).
Results: Yohimbine alone induced a dose-dependent increase in scratching, a behavioral marker of stress, but had no significant effect on grooming. Yohimbine also induced a dose-dependent increase in salivary cortisol. Pretreatment with alprazolam attenuated increases in scratching and salivary cortisol levels induced by a maximally effective dose of yohimbine (0.56 mg/kg). Attenuation of both measures occurred with similar doses of alprazolam.
Discussion: These findings suggest that modulation of the effects of a pharmacological stressor in monkeys may provide a useful approach for studying the anxiolytic effects of benzodiazepines and other compounds. Supported by USPHS grants DA11792 and RR00168.

67. Adjunctive Topiramate Therapy in Patients Receiving a Mood Stabilizer for Bipolar I Disorder: A Randomized, Placebo-Controlled Trial

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Sponsor: Samuel Gershon

Background: To investigate the efficacy and safety of topiramate vs placebo as adjunctive therapy for the outpatient management of bipolar I disorder.
Methods: In this 12-week, randomized, double-blind, placebo-controlled, parallel-group trial, adults experiencing a manic or mixed episode with a Young Mania Rating Scale (YMRS) score of ≧18 while on therapeutic levels of valproate or lithium received adjunctive topiramate or placebo. Topiramate was titrated over 8 weeks to a dosage range of 50-400 mg/day and was continued for 4 additional weeks.
Results: The average dose of topiramate after titration was 254.7 mg/day. The mean change in YMRS score from baseline was −10.1 ± 8.65 (− 40.1%) in the topiramate group (n=143) and −9.6 ± 8.17 (− 40.2%) in the placebo group (n=144, P=.797). Greater than 50% reduction in YMRS was achieved by 39% of the topiramate group and 38% of the placebo group (P=.914). No significant treatment differences were observed for secondary efficacy measures. Compared with adjunctive placebo, adjunctive topiramate did not worsen mania or induce depression. Paresthesia, diarrhea, and anorexia were more common in the topiramate group. The topiramate group achieved greater reductions than the placebo group in body weight (−2.5 vs +0.2 kg, P<.001) and body mass index (−0.84 vs +0.07 kg/m^2, P<.001).
Discussion: In patients treated with lithium or valproate to stabilize manic or mixed bipolar episodes, there was no difference in the reduction of YMRS scores between the topiramate and the placebo group. Both groups showed declines of approximately 40%. Topiramate reduced body weight significantly relative to placebo, without worsening depressive or manic symptoms compared with placebo.

68. Is the Association of PTSD and Poor Health Due to a Common Familial or Genetic Factor?

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Background: Evidence suggests that posttraumatic stress disorder (PTSD) is associated with both objective and subjective indices of poor health, but studies have typically used clinical samples with limited adjustment for confounders.
Methods: A community sample of 1852 twin pairs (University of Washington Twin Registry) was assessed for symptoms of PTSD (Impact of Events Scale) and self-reported global health status (a single 5-level question from the SF-36). An ordinal logistic regression model estimated odds ratios (ORs) for the association between the Impact of Event Scale and health status. A within-pair analysis assessed confounding by familial and genetic factors and adjusted for the possible confounding influence of age, sex, race, education and a self-reported physician diagnosis of depression.
Results: The Impact of Events Scale was strongly and significantly associated with self-reported health. Twins with scores in the highest quartile were nearly twice as likely to report poorer health than those in the lowest quartile (OR = 1.8, 95% CI 1.5-2.2). This association remained significant in the within-pairs analysis but was attenuated

(OR = 1.3, 95% CI 1.0-1.7). After further adjustment for sociodemographics and depression, the within-pair analysis was no longer significant (Ptrend <0.17). When the analysis was done separately by zygosity, evidence of differential effects in monozygotic or dizygotic pairs were not observed.
Discussion: These findings suggest that the association of PTSD with poor health is, in part, due to familial confounding as well as sociodemographic factors. Little evidence was found of confounding by genetic factors. Our findings have public health implications for intervention and prevention efforts that address the poor health of patients with PTSD.

69. Blockade of Angiotensin II AT1 Receptors Decreases Anxiety and Prevents the Isolation Stress-Induced Decrease in Cortical CRF1 Receptors and Benzodiazepine Binding
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Sponsor: Dennis Murphy

Background: Angiotensin II contributes to regulate the sympathetic and neuroendocrine systems and is an important stress hormone. During stress there is an activation of the brain and peripheral Angiotensin II systems, linked to the central and peripheral sympathetic activation. The well-known physiological actions of Ang II, including its role in the regulation of CRF secretion, are dependent on AT1 receptor stimulation. Blockade of central AT1 receptors prevents the hormonal and sympathoadrenal response to isolation stress. We asked the question whether or not AT1 receptor blockade modifies the response of higher regulatory centers during isolation, and we studied the effects of AT1 receptor antagonism on cortical CRF receptors and benzodiazepine binding.
Methods: The central and peripheral AT1 receptor antagonist candesartan was administered subcutaneously to Wistar Hannover rats at a dose of 1 0.5 mg/kg/day for two weeks, and the animals were isolated in metabolic cages for twenty four hours before sacrifice. CRF receptors and benzodiazepine binding were measured by quantitative autoradiography. In another experiment, rats were studied in the elevated plus-maze to determine anxiety-related behavior.
Results: Isolation stress decreased the number of CRF1 receptors and the benzodiazepine binding in the rat cortex. Pretreatment with the AT1 receptor antagonist completely prevented these changes. Candesartan treatment also increased the percent of time spent in the open arms of the plus-maze.
Discussion: Our results indicate that central antagonism of AT1 receptors prevents the alterations in CRF and benzodiazepine binding which occur during isolation stress. In addition, candesartan is anxiolytic. Our results implicate a modulation of cortical CRF1 receptors and the GABAA complex as molecular mechanisms responsible for the anti-anxiety effect of centrally-acting AT1 receptor antagonists. AT1 receptor antagonists may be useful in the treatment of stress-related disorders and anxiety.

70. Antimanic Response to Aripiprazole with High vs. Low Agitation
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Sponsor: Jerrold Rosenbaum

Background: Agitation is commonly associated with mania and is often a determinant of hospitalization. An analysis was carried out to compare the antimanic effects of aripiprazole, an atypical antipsychotic with low sedation effects, and placebo in acutely manic bipolar I disorder patients that were demonstrating either high or low agitation.
Methods: Data were pooled from two clinical trials of subjects diagnosed with acute manic or mixed episodes (bipolar I disorder) and randomized to either 30-15 mg/d aripiprazole (n = 259) or placebo (n = 253). The duration of both trials was three weeks. For this analysis, patients were sub-divided into two groups with either high or low agitation. High agitation was defined as a baseline PANSS Excited Component (PEC) score of > 14 and a score of > 4 on a least one PEC item (excitement, hostility, tension, uncooperative, poor impulse control). Dependent measures were change in the Young Mania Rating Scale (YMRS) total score, change in the Clinical Global Impressions-Bipolar (CGI-BP) mania score, and the PEC total score. Treatment differences (aripiprazole vs. placebo) were evaluated within the high and low agitation groups for each dependent measure. Because high agitation patients had significantly worse baseline symptoms, analyses adjusting for baseline YMRS, CGI-BP, and PEC scores were also performed.
Results: In both the high and low agitation groups, aripiprazole-treated patients showed significant decreases on the YMRS and significant improvement on the CGI-BP compared to patients on placebo at endpoint (mean difference: YMRS ARI high vs. PLA high = -6.7, ARI low vs. PLA low = -4.2; CGI-BP ARI high vs. PLA high = -0.8, ARI low vs. PLA low = -0.6; $p < 0.05$ for all). After adjusting for baseline YMRS and CGI-BP scores, aripiprazole-treated patients in both the high and low agitation groups showed significant improvements compared to placebo-treated patients in either group at endpoint ($p = 0.0016$) Additionally, in the high agitation group, aripiprazole-treated patients showed significant decreases in the PEC score compared to placebo-treated patients at endpoint (mean difference: PEC ARI high vs. PLA high = -2.6, $p < 0.05$; ARI low vs. PLA low = -1.3, $p > 0.05$, ns). After adjusting for baseline PEC score, patients on aripiprazole in the low agitation group showed significant PEC decreases compared to placebo patients ($p = 0.0145$).
Discussion: Acutely manic bipolar I disorder patients with a high level of agitation showed significant improvements in manic and agitation symptoms, as well as overall clinical impression, when treated with aripiprazole compared to placebo. Patients with low agitation also benefited from aripiprazole, although the effects on agitation symptoms were evident only after controlling for the baseline agitation level. The findings demonstrated that aripiprazole is an effective antimanic treatment for patients with bipolar 1 disorder (manic or mixed), regardless of agitation status at baseline.

71. Paroxetine Treatment of Compulsive Hoarding
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Background: Compulsive hoarding, found in many patients with obsessive-compulsive disorder (OCD), has been associated with poor response to serotonin reuptake inhibitor (SRI) medications in some, but not all, prior reports. However, no prior study has quantitatively measured response to standardized pharmacotherapy in compulsive hoarders. We sought to determine prospectively whether compulsive hoarders would respond as well as non-hoarding OCD patients to the SRI paroxetine, and whether they would have different rates of study dropout.
Methods: 79 patients with OCD (32 patients with the compulsive hoarding syndrome and 47 patients with OCD who did not report hoarding as a prominent symptom) were treated openly with paroxetine (mean dose 41.5 +/- 12.7 mg/day; mean duration 76.2 +/- 26.3 days) according to a standardized protocol. All subjects were free of psychotropic medication for at least 4 weeks prior to study entry. No psychotherapy or psychotropic medications except paroxetine were allowed during the study period. Subjects were assessed before and after treatment with the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS), Hamilton Depression Rating Scale (HDRS), Hamilton Anxiety Scale (Ham-A), Global Assessment Scale (GAS), and Clinical

Global Impression/Improvement (CGI) scale. "Response" to treatment was defined as a ≥ 35% drop in Y-BOCS score and a CGI rating of "much improved" or "very much improved." For a subset of 25 patients, the severity of compulsive hoarding symptoms was quantified before and after treatment, using the UCLA Hoarding Severity Scale (UHSS).
Results: Mean pre-treatment Y-BOCS, HDRS, Ham-A, and GAS scores were nearly identical in the two groups. There was no significant difference between groups in paroxetine dose or duration. The proportion of patients who completed treatment in each group was very similar (78% of hoarders vs. 85% of non-hoarding OCD patients). Both compulsive hoarders and non-hoarding OCD patients improved substantially with treatment, with nearly identical changes in Y-BOCS, HDRS, Ham-A, and GAS scores. Each group showed a mean 23% decrease in Y-BOCS scores with treatment. There were no significant differences between groups in the proportion of patients who met criteria for response to treatment. In the subset of patients in whom hoarding severity was assessed before and after treatment, UHSS scores decreased 26%.
Discussion: Compulsive hoarders responded equally as well to paroxetine treatment as non-hoarding OCD patients. Hoarding symptoms improved as much as other OCD symptoms. These results are consistent with several previous studies that found no association between hoarding and poor response to pharmacotherapy and suggest that SRI medications may be as effective for compulsive hoarders as for non-hoarding OCD patients. Placebo-controlled trials of SRI medications for the compulsive hoarding syndrome are now warranted.

72. The Nucleus Accumbens as Target for Deep Brain Stimulation for Treatment Refractory Depression

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Background: Recently, the results of deep brain stimulation (DBS) close to the subgenual cingulate region cg25 (Brodmann area 25) in six patients with refractory major depressive disorder were reported by Mayberg and colleagues (1). The authors chose this target on the basis of their previous findings that this region is implicated in acute stimulus-induced sadness, is metabolically overactive in treatment-resistant depression, and that clinical improvement after pharmacotherapy, psychotherapy, or limbic leucotomy is correlated with decreases in its metabolic activity. After 2 months of stimulation, five patients responded; four maintained a response after 6 months. The main focus of studies on the underlying neurobiology of major depression has focused on the description of biological differences between patients and healthy subjects such as alterations of monoaminergic or endocrine systems. The relative importance of the various biological changes has not been elucidated, correlation with specific symptoms of the disease has rarely been attempted (2).
Methods: A core symptom of major depression is anhedonia (decreased drive and reward for pleasurable activities), anxiety, and reduced motivation. The brain reward system consists of the neural pathways involved in eliciting rewarding experiences in animals and humans. Its structures, the striatum (particularly the ventral striatum or nucleus accumbens (NAcc) and amygdala, are important in emotional memory, and could as a result mediate those symptoms (3). This makes the NAcc another particularly interesting stimulation site for DBS in patients suffering from intractable major depressions. We report on first results of unilateral stimulation of the NAcc in one patient; data of four more patients will be presented in the poster. This patient was in an episode of very treatment refractory depression for 7 years, he suffered from comorbid obsessive-compulsive and other anxiety symptoms.
Results: No symptoms of hypomania, anxiety or other psychiatric symptoms were observed in the first ten days after initiation of chronic stimulation with 2 volts at 130Hz. Clinically his motivation and drive improved drastically immediately after stimulation, his mood within hours. Scores of the Profile of Mood State (POMS) were immediately before stimulation (B) and 15 minutes after stimulation (A) for sadness/anxiety 13 (B) 8 (A): thirst for action: 4 (B) 7 (A). HRSD28 ratings were 36 at baseline (B), 33 after implantation without stimulation (I), 3 one day after stimulation, 5 three days after stimulation and 18 after seven days of stimulation (S). Corresponding scores for the BDI were: 31 (B), 23 (I) and 16 (S).
Discussion: DB Stimulation of the NAcc seems to be another hypothesis guided putative approach in influencing depressive symptomatology in extremely refractory patients. It might well be that the symptoms of anhedonia and decreased drive respond to such an intervention. 1. Mayberg HS, Lozano AM, Voon V, et al. Deep brain stimulation for treatment-resistant depression. Neuron 2005;45(5):651-60. 2. Naranjo CA, Tremblay LK, Busto UE. The role of the brain reward system in depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2001;25(4):781-823. 3. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002;34(1):13-25.

73. Time Course of Depressive Symptomatology During Vagus Nerve Stimulation: Comparisons of US and European Results

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Background: An uncontrolled study of Vagus Nerve Stimulation in treatment-resistant depressive (TRD) patients in the US demonstrated positive acute (3 months) and promising long-term (12 months) results 1,2. A nearly identical study is underway in Europe; results of this study (EU) have been reported and compared to the results of the US study 3. Time course of depressive symptoms under VNS-therapy in both studies are reported and compared for the first year.
Methods: Patients with chronic or recurrent depression in a treatment-resistant, non-psychotic major depressive episode were followed over one year after being implanted with the VNS system. A priori definitions were used to define response (>50% reduction in baseline HRSD28 score) and remission (HRSD28 score <10).
Results: Until 06/05, the one-year follow-up data for 29 of the 56 total included patients in the European sample was available. The response rates of this study are compared to the US study at study 19(59%) meet criteria for response and 11 (34%) the one for remission at one year. This compares favorably with results from the US study, which showed 45% response and 27% remission at one year. The time to response was in longer in the US sample: 50% of the patients responded to VNS treatment after 7 months while in the European sample 50% of the patients responded to VNS treatment after 5 months. The group of responders increased steadily from one study visit to the other in both samples while patients in the European sample relapsed less often than in the US sample.
Discussion: As in the US sample, VNS is efficacious in a surprising number of TRD patients in the EU study. The benefit occurs in an increasing number of patients over time. Results of the EU study are somewhat better; this might be due to higher median output current settings and shorter depressive episode duration than in the US study. Differences between the US and EU studies were not only seen in the rate of response, but the response of patients in the EU sample was more sustained. 1. Rush AJ, George MS, Sackeim HA, et al. Vagus nerve stimulation (VNS) for treatment-resistant depression: A multicenter study. Biological Psychiatry 2000;47:276-286. 2. Marangell LB, Rush AJ, George MS, et al. Vagus nerve stimulation (VNS) for major depressive episodes: One year outcomes. Biological Psychiatry 2002;51:280-287. 3. Schlaepfer T, Brannan S, Frick C, et al. Response and remission after one year of vagus nerve stimulation - Comparison of US and European results. Biological Psychiatry 2005;57(8S):94S-95S.

74. Prospective Study of Plasma Stress Hormones in PTSD
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Background: Theory predicts an association between the occurrence of post-traumatic stress disorder (PTSD) and the neuro-endocrine responses to the triggering event. Alternatively, an HPA axis abnormality in PTSD may develop with time.
Methods: We examined plasma levels of cortisol, ACTH and norepinephrine (NE) in 93 male and 70 female non-injured survivors of traumatic events (123 road traffic accidents, 22 terrorist attacks and 11 others) upon admission to an emergency room (ER; 3.12±1.42 hours after a traumatic event) as well one week, one month and four months later (morning levels).
Results: At four months, 35 survivors (17 males and 18 females) had PTSD (the PTSD group) and 128 did not (the non-PTSD group). The study groups had similar age, weight, height and mean ER arrival time. The PTSD group showed higher mean levels of PTSD, anxiety and depression symptoms at all time points. The study groups had similar levels of plasma cortisol, ACTH, and NE at all time points (for non-PTSD vs. PTSD respectively: **ER levels**: cortisol 13.6±5.8 vs. 13.8±8.2; ACTH 22.1±18.7 vs. 20.6±11.8; NE 318.6±146.1 vs. 272.2±130.2; **One week levels**: cortisol 12.7±5.0 vs. 12.7±5.7; ACTH 24.5±16.5 vs. 21.6±15.8; NE 294.2±133.5 vs. 271.0±132.6. **One month levels**: cortisol 12.1±6.2 vs. 13.2±6.2. **Four month levels**: cortisol 12.0±5.3 vs. 10.7±3.9; ACTH 25.8±15.1 vs. 22.5± 10.8; NE 310.4±143.8 vs. 294.0.1±157.0). An analysis by gender revealed lower four months cortisol in males with PTSD (9.5±3.0 in PTSD vs. (11.9±4.3 in non-PTSD; t([df=86] = 2.08, p.<0.05) but not in females. Repeated measure ANOVA for morning cortisol showed significant Gender (P.<0.02) and Gender by Diagnosis (p.< 0.04) interaction.
Discussion: Early endocrine measures are poor risk indicators of PTSD. Time dependent sensitization of the HPA axis might occur in men who develop PTSD.

75. Risperidone vs. Bupropion Combined with SSRIs in Treatment Resistant Unipolar Major Depression
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Background: Treatment resistant unipolar major depression (MDD) is a common condition. Prior research suggests that augmentation or combination strategies can be effective. One such strategy has been to combine selective serotonin reuptake inhibitors (SSRIs) with atypical antipsychotics. To date, augmenting effects have been shown to occur with olanzapine and ziprasidone. To determine whether this is a class effect, we compared risperidone (RIS) vs. bupropion SR (BUP) combination with SSRIs.
Methods: Patients with MDD (n=16, 13 females, mean age 40.8, SD=10.1) who had previously failed two antidepressants including an SSRI, were randomly assigned to treatment with BUP or RIS (initial doses 150 and 1.0 mg./day respectively) combined with an SSRI. Doses were then titrated to 300 (BUP) or 3 mg./day (RIS) as tolerated. Outcome measures included the 17-item Hamilton Rating Scale for Depression (HAM-D17) and the Montgomery Asberg Depression Rating Scale (MADRS). Visits occurred at baseline and weeks 1, 2, 4, and 6.
Results: Mean maximum dose was 2.47 (SD=0.82) for RIS and 293.75 (SD=67.81) for BUP. One patient dropped out of the RIS group after week 4, none with BUP. There were no significant differences between groups on baseline to endpoint change with any outcome measure. Mean change from baseline to endpoint for the HAM-D17 was 7.4 (SD=6.7) for RIS and 5.6 (SD=7.4) for BUP, yielding effect sizes of 1.72 and 1.02 respectively. Change on MADRS was 9.4 (SD=12.4) for RIS and 8.1 (SD=8.9) for BUP, yielding effect sizes of 1.21 and 1.31 respectively. However, RIS produced a more robust effect at the end of week 1; change in HAM-D, baseline to week 1: RIS=6.8 (SD=5.7), BUP =1.3 (SD=3.8) (t=2.62, df=14, p=0.04); effect sizes: RIS=1.88, BUP=0.52. At week 1, mean HAM-D17 scores were 12.6 (SD=4.8) for RIS and 17.1 (SD=3.8) for BUP.
Discussion: Both RIS and BUP are effective combination strategies with SSRIs, but BUP produced a more rapid response. With RIS, treatment week 1 and endpoint scores were equivalent.

76. Effects of Chronic Prednisone Therapy on Mood and Memory: A Follow-Up Study
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Background: Our group previously reported greater depressive symptom severity, poorer memory and smaller hippocampal volumes in patients receiving long-term prednisone therapy than in controls with minimal prednisone exposure (Brown et al., Biological Psychiatry 55:538-545;2004). In this report, these patients and controls and those from a similar study (Brown et al., Psychosomatics 44;204-208;2003) were assessed a mean of 4 years after the first assessment to determine if mood and cognition remained stable over time or showed further decline.
Methods: A total of 13 participants (7 prednisone-treated patients and 6 controls) were identified and reassessed with the Brief Psychiatric Rating Scale (BPRS), Hamilton Rating Scale for Depression (HRSD), Young Mania Rating Scale (YMRS), Rey Auditory Verbal Learning Test (RAVLT) and Stroop Color Word Test. Follow-up MRIs for hippocampal volume analysis were available for two prednisone-treated participants and hippocampal volumes were assessed using the same methods and rater as in the original report. Cognitive data are reported as normative t-scores.
Results: Of the 13 participants, 6 were on long-term prednisone therapy at baseline and remained on prednisone at follow-up, with 2 at the same dose, 3 at a reduced dose and 1 at a higher dose. One participant on prednisone at baseline discontinued prednisone 12 months prior to follow-up. Assessment scores in both groups remained fairly stable over time although HRSD scores increased in the prednisone-treated group from a mean of 7.6+6.6 to 14.0+9.2 (p </= 0.05). Patients on prednisone had significantly (p</= 0.05) lower scores on RAVLT total words recalled (47.4+9.9 vs 59.4+7.2); higher scores on the BPRS (28.8+7.6 vs 21.0+2.0), and HRSD (14.0+9.2 vs 5.3+1.5); a trend (p>/= 0.1) on the Stroop (43.1+6.8 vs 52.6+9.7); but no significant difference on the RAVLT delayed (49.7+11.2 vs 58.6+7.9). The participant who discontinued prednisone showed decreases on the BPRS (33 to 31), HRSD (19 to 12), and YMRS (5 to 1), and improvement on the Stroop (39 to 54), and RAVLT delayed (39 to 49) from original assessment to reassessment. We had follow-up MRIs for 2 participants both taking 10 mg/day of prednisone at baseline and follow-up. One had a right hippocampal volume change of -8.5% and a left hippocampal volume change of +12.1%. The other had a right hippocampal volume change of -13.8% and a left volume of change of -3.5%.
Discussion: Depressive symptoms remained higher and, in general RAVLT and Stroop scores lower, than in controls. Although preliminary in nature, our findings suggest that months or years of prednisone exposure is associated with changes in mood, memory and hippocampal volume that appear to remain stable over time. We did not find strong evidence of an ongoing neurodegenerative process secondary to the prednisone therapy. Supported by NIH grant MH01725.

77. Effect of Two Prednisone Exposures on Mood and Declarative Memory
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Background: Corticosteroids are associated with mood changes and declarative memory impairment. The magnitude of mood change in

patients receiving prednisone has been associated with previous lifetime corticosteroid exposure, consistent with a sensitization or kindling process whereby greater effects are observed with repeated exposure. The effect of multiple corticosteroid exposures has not been previously examined prospectively. In this study, steroid-naive patients were given two brief prednisone exposures.
Methods: Thirty volunteers received (in random order) two 3-day exposures of prednisone(PRED) (60 mg/day) and one of identical placebo (PBO), with 11-day washouts (WO) between each medication exposure. Declarative memory was repeatedly assessed with the Rey Auditory Verbal Learning Test (RAVLT), and mood with the 21-item Hamilton Rating Scale for Depression, Young Mania Rating Scale and Internal State Scale before and after each 3-day prednisone/placebo exposure.
Results: A significant decrease in aspects of RAVLT performance was observed after the first prednisone exposure. The decline in RAVLT performance was significantly smaller after the second as compared to the initial prednisone exposure. No significant mood changes were found.
Discussion: A second prednisone exposure was associated with an attenuated declarative memory effect. These data suggest tolerance or habituation, rather than sensitization, to prednisone effects on declarative memory during a second exposure. Supported by the Stanley Medical Research Institute.

78. In Vivo Effects of Lithium on Synaptic Plasticity and Levels of BDNF and Phospho-CREB in Hippocampus
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Sponsor: Joseph Calabrese

Background: Molecular biological studies have shown that lithium exposure upregulates brain-derived neurotrophic factor (BDNF) and cyclic adenosine monophosphate response element-binding protein (CREB). Since BDNF and CREB play important roles in synaptic plasticity, it has been suggested that lithium may increase synaptic plasticity. However, the effects of lithium on synaptic plasticity have never been fully examined. This study examined the effects of lithium exposure on functional and morphological synaptic plasticity and correlate these findings with the effects of lithium exposure on the levels of BDNF and phospho-CREB the in the dentate gyrus (DG) and the area CA1 of the hippocampus.
Methods: LiCl at 1 mEq/kg/day (experimental group) or PBS vehicle (control group) was injected (IP) into male Sprague-Dawley rats (250-350 g) for 14 days. For functional synaptic plasticity, animals were decapitated, and hippocampal slices were prepared in a submerged-type chamber oxygenated with artificial cerebrospinal fluid (aCSF). The input and output (I/O) function and long-term potentiation (LTP) of field excitatory postsynaptic potentials (fEPSPs) were determined in the DG and the area CA1. For morphological synaptic plasticity, animals were decapitated and the brains were prepared for Golgi staining. After the staining, a Sholl analysis was performed to examine the density of dendrites and dendritc spines of the principle neurons in the DG and the area CA1. For the levels of BDNF and phospho-CREB, the brains were prepared for ELISA of BDNF and phospho-CREB.
Results: At 14 days, 1mEq lithium significantly increased both the I/O function and the LTP of fEPSPs in DG. This treatment also significantly increased the LTP of fEPSPs in the area CA1. At 14 days, 1mEq lithium significantly reduced the density of dendritic branches in the outer one-half of the dendritic trees of the granule cells in DG. This treatment also significantly reduced the density of dendritic branches in the outer 2/3 of basal dendritic trees of the CA1 pyramidal neurons. This treatment did not change the total density of dendritic spines, but reduced the density of the N subtype of spines in this region. At 14 days, 1 mEq lithium did not significantly alter the level of BDNF, but showed a tendency to increase the level of p-CREB in the DG and area CA1. These results suggest that sub-chronic exposure to lithium increased synaptic transmission and LTP in the hippocampus. These increases may not be associated with morphological changes in dendrites and spines of the principle hippocampal neurons nor the levels of BDNF and p-CREB in the DG and the area CA1.
Discussion: A neuroplastic theory largely based on molecular biological data proposes that lithium may increase synaptic plasticity, and an increase in the production of BDNF and p-CREB may be involved in this lithium-induced actions on synaptic plasticity. This study demonstrates that lithium treatment upregulates functional synaptic plasticity such as an increase in synaptic transmission and functional synaptic plasticity. These changes in synaptic plasticity could be mediated by molecular mechanisms other than those mediated by BDNF and CREB. This study suggests a caution that negative changes in morphological synaptic plasticity such as the atrophy of dendrites or dendritic spines do not always indicate impairment in synaptic activity or functional synaptic plasticity in the brain.

79. Efficacy and Tolerability of Low Doses of Paroxetine CR in Elderly Outpatients with Major Depression
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Sponsor: Russell Poland

Background: Late-onset depression is recognized as a significant public health concern, particularly in the face of an aging population. There have been very few studies reported in the literature assessing the efficacy and safety of SSRI's in elderly depressed patients. Due to a high placebo response in this population only few of these studies were able to demonstrate antidepressant efficacy. Dose response effect is typically assessed by administering fixed doses of the test drug in multiple study arms. To our knowledge this is the first clinical study to report the efficacy and safety profile of low fixed doses of SSRI in the elderly depressed population. The aim of this study is to evaluate the efficacy and safety of two different doses of Paroxetine CR in the treatment of elderly patients with major depression disorder.
Methods: 528 outpatients with major depression disorder (age 60-91; mean 67) with a Hamilton depression score or ≥ 18 (HAM-D 17 item) were randomly assigned to 10 weeks of fixed doses of Paroxetine CR 12.5 mg or 25 mg or placebo. The primary efficacy variable was HAM-D total score mean change from baseline to end point.
Results: Baseline-endpoint change for drug vs placebo demonstrated efficacy for both Paroxetine 12.5mg (-1.8;[95% C.I. -3.41, -0.19]; p=0.029) and 25mg (-3.3; [95% C.I. -4.84 -1.68]; p = <0.001) as measured by the primary variable (HAM-D [LOCF]). Remission rates (HAM-D total score ≤ 7 at endpoint) were 33% for Paroxetine CR 12.5mg (p = 0.280) and 41% for Paroxetine CR 25 mg (p = 0.008) relative to placebo (28%). Efficacy was demonstrated for Paroxetine CR 12.5 mg and 25 mg by the LOCF CGI-S and the HAM-D depressed mood item. Both active treatment groups had a low incidence of AEs. There was a low incidence of withdrawals due to AEs (12.5 mg = 6%; 25 mg = 8%; placebo = 7%).
Discussion: These data confirm the effectiveness of Paroxetine CR in late-life depression and underscore the utility of 12.5mg and 25mg regimens in this population. Because the data suggest a dose-response effect, patients who initiate treatment at 12.5mg daily without response, may progress to 25mg daily while retaining a favorable safety profile.

80. Efficacy of Ziprasidone in Dysphoric Mania: Pooled Analysis of 2 Double-Blind Studies
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Background: Dysphoric mania is defined as a manic episode that includes symptoms of depression and irritability but which does not satisfy full criteria for a major depressive eipsode.(1) Nonetheless

these patients have clinically relevant depressive symptoms, and may be at an elevated risk for suicide, making effective treatment imperative. Ziprasidone has demonstrated efficacy in bipolar manic patients including those with mixed mania; therefore, it is of interest to determine whether monotherapy with ziprasidone is effective in the subpopulation of patients with dysphoric mania.
Methods: To evaluate the potential efficacy of ziprasidone in treating patients with dysphoric mania, data were pooled from 2 similarly designed, randomized, double-blind, 3 week placebo-controlled bipolar mania trials. (2,3) Dysphoric mania was defined as a score of >2 on >2 of the following items of the SADS-C: 1-6, 16, and 20 (extracted HAM-D). Change in depressive symptoms were measured by the HAM-D on days 2,4,7,14, and 21 and evaluated by an MMRM analysis (fixed effects for treatment and protocol and random effects for visit and subject). Additional assessments included changes in MRS, CGI, PANSS, and GAF.
Results: HAM-D for ziprasidone was significantly superior to placebo at all visits starting on day 4 (P=0.026). Mean change from baseline to 21-day endpoint was -4.2 (SEM0.7) for the ziprasidone group vs. -2.1 (SEM 1.0) for the placebo group (P = 0.027).
Discussion: This analysis of pooled data from 2 randomized, double-blind, placebo-controlled studies demonstrated thawt treatment with ziprasidone monotherapy significantly improved depressive symptoms, assessed by the extracted HAM-D, in patients with dysphoric mania. Response to ziprasidoe was rapid (onset by day 4) and sustanined through 3 weeks.

81. A Single-Blind Prospective Study of Quetiapine for the Treatment of Mood Disorders in Adolescents Who Are at High Risk for Developing Bipolar Disorder

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Background: Offspring of bipolar parents have an elevated risk for developing bipolar disorder. Since the clinical manifestations of bipolar disorder often begin early in life and may worsen with age, it is imperative that this illness is recognized and treated as early as possible. Bipolar disorder may have a number of prodromal or early onset manifestations, including cyclothymia, depression, and subsyndromal symptoms of mania. Controlled investigations of atypical antipsychotics suggest that they are effective and well tolerated for the treatment of mania and depression in adults with bipolar disorder. Quetiapine is effective as adjunctive treatment and monotherapy for adolescent mania. Although there are several studies suggesting that divalproex may be effective for the treatment of mood symptoms in children at familial risk for developing bipolar disorder, to our knowledge, there have been no studies examining the use of atypical antipsychotics for the treatment of mood syndromes in adolescents at familial risk for developing bipolar disorder. The objective of this study was to determine the effectiveness and tolerability of quetiapine for the treatment of mood disorders other than bipolar I disorder in adolescents with at least one parent with bipolar disorder.
Methods: Twenty adolescents (ages 12-18 years) with a parent with bipolar I disorder and diagnosed with a mood disorder other than bipolar I disorder (dysthymia, major depressive disorder, depressive disorder not otherwise specified, cyclothymia, bipolar II disorder, or bipolar disorder not otherwise specified) participated in a 84-day single-blind (rater-blind) study of quetiapine (300-600 mg/d). Patients were included if their baseline Young Mania Rating Scale (YMRS) score was > 12 or Childhood Depression Rating Scale (CDRS) scores were > 28. Effectiveness and tolerability assessments were performed on Days 0, 7, 14, 21, 28, 42, 56, 70, and 84 or endpoint. Primary efficacy measures were change from baseline to endpoint in YMRS and CDRS scores. Tolerability measures included documentation of adverse events, vital signs, laboratory measures, and movement scales.
Results: Twenty adolescents [age, mean (SD)=14.2 (1.8) years, sex, females, N (%)= 8 (40), race, White, N (%)=2 (90)] were initiated on 100mg of quetiapine on day 1 and titrated to 400mg by day 4 followed by flexible dosing within a range of 300-600mg/day [endpoint dose, mean (SD)=460 (86) mg/day, median=400 mg/day]. Eleven (55%) of the adolescents were diagnosed with BP NOS, 3 (15%) with dysthymia, 3 (15%) with bipolar II disorder, and 2 (10%) with cyclothymia, and 1 (3.3%) with major depressive disorder. Five adolescents discontinued study participation prior to day 84; 1 (5%) for lack of response, 1 (5%) for symptom exacerbation, and 3 (15%) for withdrawal of consent/assent. Baseline mean (SD) YMRS and CDRS scores were 18.1 (5.5) and 38.2 (9.8), respectively. Endpoint mean (SD) YMRS and CDRS scores were 8.1 (8.5) and 27.4 (9.0). YMRS and CDRS scores significantly decreased from baseline to endpoint ($t=-6.0$, $p<0.0001$ and $t=-4.1$, $p<0.0006$). The most common side effects were sedation (N=13, 65%), headaches (N=8, 40%), and dry mouth (N=8, 40%), which were generally rated as mild.
Discussion: The results of this study suggest that quetiapine may be effective for the treatment of adolescents with mood disorders other than bipolar I disorder and a familial risk for bipolar disorder. Further double-blind placebo-controlled studies of quetiapine in this population are needed.

82. Manic Symptoms During Bipolar Depression: Impulsivity and Suicidality

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Background: In contrast to extensive literature on the frequent occurrence of depressive symptoms in manic patients, there is little information about manic symptoms in bipolar depressions. Impulsivity is a prominent component of the manic syndrome, so manic features during depressive syndromes may be associated with impulsivity and its consequences, including increased risk of substance abuse and suicidal behavior. Therefore, we investigated the incidence of manic symptoms and their relationships to impulsivity and clinical characteristics in patients meeting criteria for bipolar depressive episodes.
Methods: In 56 bipolar depressed subjects, we investigated the presence of manic symptoms, using Mania Rating Scale (MRS) scores from the Schedule for Affective Disorders and Schizophrenia (SADS), and examined its association with other psychiatric symptoms (depression, anxiety, and psychosis), age of onset, history of alcohol and/or other substance abuse and of suicidal behavior, and measures of impulsivity.
Results: MRS scores ranged from 0 to 29 (25th-75th percentile range 4-13). Mania scores correlated significantly with anxiety and psychosis scores, but not with depression, suggesting the superimposition of a separate psychopathological mechanism. Measures of impulsivity and history of substance abuse, head trauma, or suicide attempt increased with increasing mania scores. Depressed subjects with mania scores higher than 9 had earlier onset of illness, had higher ratings for anxiety, and were significantly more likely to have histories of alcohol abuse, head trauma, and attempted suicide, than subjects with lower mania scores. Impulsivity, both trait (Barratt Impulsiveness Scale (BIS) scores) and state-like (impulsive performance on a modified continuous performance test, the Immediate and Delayed Memory Task (IMT-DMT)), was elevated in these subjects.
Discussion: Manic symptoms during bipolar depressive episodes were associated with greater impulsivity, and with histories of alcohol abuse and suicide attempts. Manic symptoms during depressive episodes suggest the presence of a potentially dangerous combination of depression and impulsivity.

83. Galanin-Evoked Reductions in Serotonergic Neurotransmission in the Hippocampus and Dorsal Raphe Nucleus Are Attenuated by GAL_3 Receptor Antagonists

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Sponsor: Theresa Branchek

Background: Several studies have demonstrated the ability of galaninergic peptide neurotransmission to modulate central 5-hydroxytryptamine (5-HT) function (Misane et al., 1998; Xu et al., 1998; Kehr et al., 2002, Yoshitake et al., 2003), thereby potentially linking galanin signaling to depression (Lu et al., 2005). In this light, the potent and selective GAL_3-antagonist SNAP 37889 has recently been shown to be effective in acute and chronic animal models of anxiety and depression (Blackburn et al, ACNP, 2005). Here we have tested the hypothesis that galanin's inhibitory effects on 5-HT levels in the ventral hippocampus and neuronal activity in the dorsal raphe nucleus are Gal_3-mediated.
Methods: All experiments were performed on male rats according to modifications of previously published protocols.
Results: Administration of galanin (1.5 nmol/ 1000 nl) into the lateral ventricle decreased basal 5-HT levels in the ventral hippocampus for up to 3 hours (AUC=58% baseline values) when measured using *in vivo* microdialysis. Oral administration of the Gal_3 antagonist SNAP 37889 (30mg/kg, p.o.) did not, itself, significantly alter 5-HT levels over a 2h observation period. However, 2h after the administration of SNAP 37889, the maximal inhibitory effect of galanin on hippocampal 5-HT output was reduced by 43%. As expected, pretreatment with the 5-HT_{1A} receptor antagonist, WAY100635 (0.6 mg/kg, s.c., 2 h), also reduced galanin's effect (33% reduction). When co-administered, the antagonistic effects of the GAL3 and 5-HT1A receptor antagonists were additive, reducing the effect of galanin by 77%. These results indicate that the effect of galanin on hippocampal 5-HT release is mediated directly, via GAL_3 activation, as well as indirectly, via release of 5-HT and subsequent activation of 5-HT_{1A} autoreceptors.Complementary experiments were performed using electrophysiological approaches. Due to limited solubility characteristics of SNAP 37889, a second GAL_3 antagonist, SNAP 398299, was used for electrophysiology (K_i GAL_3=5nM; K_i $GAL_{1,2}$ > 10000 nM). Galanin (0.1-5 nmol, icv) elicited a dose-dependent decrease in neuronal firing in the dorsal raphe nucleus (DRN) recorded *in vivo* using extracellular recordings in anesthetized rats. The maximal inhibition was found at 1.5 nmol. SNAP 398229 produced a dose-dependent reversal of the maximum galanin-mediated reduction in DRN neuronal firing (1-8 mg/kg cumulative dose, i.v.). Moreover, in a DRN slice preparation, galanin increased potassium channel conductance in approximately 37% of neurons tested. SNAP 398299 significantly blocked this galanin-mediated increase by approximately 50% in responding neurons.
Discussion: Taken together, the results of these studies suggest that GAL_3 receptors at the level of the DRN may be stimulated by galanin to negatively modulate central 5-HT neurotransmission. These findings provide potential mechanistic insight into the anxiolytic and antidepressant actions of GAL_3 antagonists. Altered tone of the galanin system in affective disorders such as depression or anxiety may be an underlying substrate for treatment.

84. Decreased Serotonin-1A Receptor Binding in Social Anxiety Disorder

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Sponsor: Past Travel Awardee, ECNP-ACNP Fellow, 2003

Background: Using positron emission tomography (PET) and [^{11}C]WAY-100,635 we had previously demonstrated a negative correlation between serotonin-1A (5-HT_{1A}) receptor binding potential (BP) and trait anxiety in healthy subjects (Tauscher et al. 2001). Furthermore, a significant decrease of 5-HT_{1A} binding had been demonstrated in patients suffering from panic disorder (Neumeister et al. 2004). The aim of this study was to explore 5-HT_{1A} BP in social anxiety disorder (SAD).
Methods: We recruited 10 patients suffering from SAD (mean age 33.3 ys. ± 10.5 SD) and 18 healthy male subjects (mean age 28.2 ys. ± 8.3 SD). Dynamic PET scans were acquired after bolus injection of [^{11}C]WAY-100,635. We collected a series of 30 time frames during 90 minutes. 35 contiguous slices (matrix 128x128) with a slice thickness of 4.25mm (FWHM = 4.36mm) were reconstructed. T1-weighted MR images were coregistered to the PET images. Eight regions of interest (anterior cingulate cortex, insula, orbitofrontal cortex, amygdala, hippocampus, raphe nucleus, motor cortex, cerebellum) were drawn on coregistered anatomical images. The raphe region was identified on PET images. For quantification of 5-HT_{1A} BP we used a Simplified Reference Tissue Model (SRTM) based on a two-tissue compartment model with the cerebellum as reference region (Lammertsma et al. 1996).
Results: The 5-HT_{1A} BP was significantly lower in the SAD patients as compared to healthy controls in the anterior cingulate cortex (mean ± SD: SAD=3.1 ± 1.0; HC=4.1 ± 0.9; p= .010), insula (SAD=3.5 ± 1.2; HC=4.8 ± 1.2; p= .007), orbitofrontal cortex (SAD=3.0 ± 1.0; HC=3.7 ± .9; p= .035), amygdala (SAD=3.0 ±1.1; HC=4.1 ± 1.1; p= .009), and raphe nucleus (SAD=1.4 ± 0.6; HC=2.1 ± .6; p= .009). There was no significant correlation between 5-HT1A BP and age, or any radiochemical variables.
Discussion: To our knowledge, this is the first [^{11}C]WAY-100,635 PET study demonstrating a relatively lower 5-HT_{1A} binding potential in social anxiety disorder patients as compared to a healthy control group. These findings are in line with animal models in rodents showing increased anxiety-like behaviour in 5-HT_{1A} knock-out mice (Parks et al. 1998), and previous studies in man showing a negative correlation between 5-HT_{1A} binding and trait anxiety in healthy volunteers (Tauscher et al. 2001), and decreased 5-HT_{1A} binding in panic disorder (Neumeister et al. 2004).

85. Using Symptom Improvement Patterns to Predict Remission in Depressed Outpatients Treated with Venlafaxine Extended Release (XR) or Selective Serotonin Reuptake Inhibitors (SSRI)

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Background: Less than half of patients treated with an antidepressant reach remission. Patients who remit early in treatment may not require a change in treatment, but differentiating between patients who will achieve remission later from those who will not remit at all may improve outcomes. This sub-analysis of a 180 day, rater-blinded open-label study in depressed outpatients was designed to examine the patterns of symptom improvement in the first four weeks of treatment and how these patterns relate to remission with venlafaxine XR or SSRIs.
Methods: Outpatients (n=1385) with major depressive disorder (MDD) and a total score of ≥20 on the 17-item Hamilton Depression

Rating Scale (HAM-D17) were randomly assigned to receive venlafaxine XR 75-225mg/day (n=688) or an SSRI (n=697): fluoxetine 20-80mg/day, paroxetine 20-50mg/day, citalopram 20-40mg/day, and sertraline 50-200mg/day. Remission rates for venlafaxine XR and SSRIs were compared at 90 and 180 days. Remission was defined as a HAM-D17 total score ≤7. The three symptom domains evaluated were: 1) mood, 2) psychic anxiety, and 3) somatic symptoms. A fourth combined anxiety and somatic symptom domain was also evaluated. Patient change scores on the above symptom domains during the baseline to day 14, day 14 to 30, and baseline to day 30 treatment periods were compared with remission status at day 90 and 180.
Results: Ninety day remission rates were 35.0% (193/552) and 29.5% (163/553) for venlafaxine XR and SSRIs, respectively; this difference was not statistically significant. The predictors that best distinguish between remitters and non-remitters (at day 90) for venlafaxine XR treated patients are the day 14-30 mood (P =0.0006) and somatic symptom (P =0.0005) domain change scores, while the day 14-30 somatic (P =0.0052) domain change score was the best predictor for the SSRIs. For both treatment groups baseline to day 30 mood and somatic domain change scores were also significant predictors of 90 day remission (P<0.0001).
Discussion: Although cross-sectional remission rates for the two treatment groups did not differ significantly, the pattern of symptom improvement did. Patients remitting on venlafaxine XR at 90 day may see an improvement in the first 2-4 weeks of treatment on the mood and somatic symptom domains, while patients remitting on an SSRI may see an improvement on the somatic domain only. These data provide preliminary evidence from a real world treatment setting of differing patterns of symptom improvement that may help predict which patients will achieve remission with antidepressant agents that involve different neurotransmitter systems.

86. Inhibited Temperament and HPA Axis Function in Healthy Adults

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Personality traits such as neuroticism and behavioral inhibition have been implicated in the etiology of mood and anxiety disorders. These traits are more common in the families of individuals with major depression and anxiety disorders, respectively, and are prospective predictors of the development of these disorders. Neuroticism and inhibition may confer or reflect sensitivity to negative stimuli or stressors. Such temperamental features are of particular interest because childhood adversity and adult stressors are closely linked to the development of major depression. Several lines of research have documented hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in patients with major depression as well as in children and monkeys with inhibited temperaments. Cortisol response to the dexamethasone/corticotropin-releasing hormone (Dex/CRH) test has been shown to be highly correlated with levels of neuroticism in a sample of healthy adults. We previously reported results from a pilot study of healthy adults showing that cortisol response to the DEX/CRH test was negatively associated with novelty seeking. The present investigation tested the hypothesis that stress-reactive temperaments would be predictive of cortisol hyperactivity in the Dex/CRH test in a larger sample of healthy adults.
Methods: Fifty-eight adults completed diagnostic interviews and questionnaires assessing personality domains and symptoms of anxiety and depression. The Dex/CRH test, a standardized neuroendocrine challenge protocol which combines oral dexamethasone pretreatment with intravenous CRH infusion, was performed on a separate visit.
Results: Harm avoidance was a significant positive predictor of cortisol response to the Dex/CRH test, while novelty seeking was a significant negative predictor of cortisol concentrations in this test.
Discussion: These results replicate and extend the findings of our pilot study and suggest that temperamental sensitivity to negative stimuli is associated with HPA axis hyperactivity. Dysfunction of the HPA axis, which has previously been linked to major depression and anxiety disorders, may account for the association between personality traits and these disorders.

87. Antidepressant Treatment and Risk of Suicide Attempt by Adults with Major Depressive Disorder: A Propensity-Adjusted Retrospective Cohort Study

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Sponsor: Robert Gibbons

Background: In October 2004, the U.S. Food and Drug Administration (FDA) directed manufacturers of all antidepressant drugs to revise the labeling for their products to include a boxed warning and expanded warning statements that alert health care providers to an increased risk of suicidality (suicidal thinking and behavior) in children and adolescents being treated with these agents. Recently, FDA began a review of available data to determine whether an increased risk of suicidality may exist for adults.
Methods: The objective of this ongoing study is to examine the potential relationship between antidepressant treatment and suicide attempts among adults (aged 19 or older) with major depressive disorder (MDD), using a national community sample of managed care enrollees. A retrospective longitudinal cohort was created using paid insurance claims from the PHARMetrics Integrated Outcomes Database for all health care services and prescription fills for adults who were newly diagnosed with MDD between January 1997 and March 2003 and had at least one year of pre-index data and six months of follow-up data. The primary outcome measure of the study was suicide attempt, as indicated by ICD-9 or 10 codes in any health care setting. To test the hypothesis that antidepressant use (defined as either: selective serotonin reuptake inhibitor, tricyclic antidepressant, other antidepressant, multiple antidepressants, or no antidepressant) affected the risk of suicide attempt in these observational data, the groups were first matched using propensity scores and then compared using a stratified Cox regression analysis. Propensity score matching allows us to match the five antidepressant use groups on a large number of potential confounders (e.g., year, sex, age at MDD diagnosis), then stratify the sample into quintiles that are homogenous in terms of the likelihood of using a particular antidepressant. Subsequent statistical comparisons of time to suicide attempt between drug and non-drug conditions can then be made within and across the propensity score matched strata.
Results: 190,797 subjects met inclusion criteria (71% female; mean age 43.3 years at MDD diagnosis). 485 suicide attempts were recorded in the cohort across the follow-up period (median follow-up time 1.2 years, range 0.5-5.7 years). Crude overall suicide attempt rates were 0.18% per year or 180 per 100,000 in this MDD cohort. Nearly 3 times the risk was observed in the first 8 weeks after diagnosis (0.033% per month) relative to months 2-6 (0.011% per month), and almost 5 times the risk relative to 6 months or more (0.007% per month), indicating that the hazard of a suicide attempt decreases over time after a new diagnosis of MDD. Results of the propensity score matched comparisons of antidepressant effects on suicide attempt rates will be presented along with methodologic and statistical issues related to the analysis of rare event data and analysis of large integrated healthcare databases.
Discussion: To date, analyses of suicide rates have been largely restricted to meta-analyses of suicidal ideation, national cohort studies comparing rates of suicide versus rates of antidepressant medication use, and county-level suicide rates versus antidepressant prescription rates. Person-level analyses have generally not been possible due to the low base-rate of suicide and the lack of availability of large inte-

grated databases. Results of analysis of this large integrated database on antidepressant utilization and suicide attempts will clarify the possible relationship.

88. Mitochondrial Related Gene Expression In Suicide

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Background: Suicide affects approximately 5-15% of individuals with mood disorders and is a leading cause of death for males under 40 years of age[(1)]. The neurobiology of suicide and mood disorders is not understood, and may involve alterations in limbic-cortical brain circuits. A neurobiological predisposition to alterations in circuitry might in part have genetic components. Family studies are difficult to conduct, and case-control genetic studies require identification of candidate genes. Microarrays allow for the simultaneous analysis of gene expression patterns in multiple brain regions for thousands of gene targets. One previous microarray analysis of suicide showed that gene expression differences were not significantly different in monoaminergic mediated pathways compared to controls in prefrontal cortical regions[(2)].
Methods: Mitochondrial related gene expression classes (mitochondrial genes encoded by nuclear DNA, and proteasome, chaperone, reactive oxygen stress, and apoptotic genes) were investigated in major depressive disorder (MDD) and bipolar disorder (BPD) subjects that died by suicide compared to MDD and BPD subjects that died by other causes. Cases were included in the microarray analysis that had no agonal factors, brain tissue pH => 6.6, RNA quality => 1.4 by Agilent, and agreement between two post-hoc microarray measures for chip similarity. Aging and pH are known to significantly correlate to gene expression and were used as a continuous covariate in downstream analysis of mitochondrial related gene expression.
Results: Few mitochondrial and related genes were dysregulated in BPD-suicide or MDD-suicide groups. There was minimal overlap in differential expression of mitochondrial-related genes between each separate suicide group (BPD or MDD). Five mitochondrial related pathways were not significantly over-represented in suicide. However, specific mitochondrial related genes were dysregulated in the amygdala-anterior cingulate regions of suicide groups. For all genes, BPD-suicide showed larger numbers of dysregulated genes in the anterior cingulate cortex, DLPFC, and amygdala compared to MDD-suicide.
Discussion: The results implicate specific genes in mitochondrial related pathways in the predisposition of suicide as worthy of further consideration. Specific genes were differentially expressed in mitochondrial and related classes (apoptosis, chaperone, proteasome, and reaction to oxygen stress) in one part of the limbic circuit involving the anterior cingulate and amygdala. The preliminary results suggest that more genes are dysregulated in individuals with BPD that commit suicide compared to MDD that commit suicide. More genes were dysregulated in limbic regions compared to the DLPFC in both suicide groups. These genes require confirmation by independent replication and validation studies. The alteration of specific candidate genes in limbic circuitry in BPD and MDD is a promising approach for study of the neurobiology involved in the predisposition to suicide. *The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium.* 1. Gwadry, F.G., Sequeira, A., Hoke, G., Ffrench-Mullen, J.M.Turecki, G., Molecular characterization of suicide by microarray analysis. Am J Med Genet C Semin Med Genet, 2005. 133(1): p. 48-56. 2. Sibille, E., Arango, V., Galfalvy, H.C., Pavlidis, P., Erraji-Benchekroun, L., Ellis, S.P., et al., Gene expression profiling of depression and suicide in human prefrontal cortex. Neuropsychopharmacology, 2004. 29(2): p. 351-361.

89. Altered Sensitivity of Peripheral Blood Leukocytes to Glucocorticoids in Deployment-Related Postraumatic Stress Disorder

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Sponsor: Herman M van Praag

Background: Studies have linked traumatic stress exposure and posttraumatic stress disorder (PTSD) to various somatic conditions including chronic fatigue and musculoskeletal disorders. Clinical studies have suggested the biological pathways through which these stress-related disorders may be expressed. Since altered sensitivity to glucocorticoids has been observed in both PTSD as well as in patients with medical conditions like chronic fatigue syndrome, we hypothesized that changes in leukocyte GR function can be a common factor in both conditions.
Methods: We recruited 26 non-medicated male patients with deployment-related PTSD and 26 trauma controls (TC), matched for age, region and year of deployment at the Central Military Hospital and the Dutch Veterans Institute. We assessed PTSD symptoms by the clinician administered PTSD symptom scale (CAPS) and fatigue by using the checklist individual strength (CIS-20). We analyzed peripheral blood for glucocorticoid receptor (GR) function by in vitro measurement of lymphocyte sensitivity to dexamethasone. Affinity and number on peripheral blood mononuclear cells (PBMC) were determined with a whole cell competitive GR binding-assay using 3H-dexamethasone.
Results: PTSD patients scored significantly higher on the CIS-20 questionnaire in comparison to TC ($p<0.001$). Inhibition of T cell proliferation by dexamethasone was significantly lower in cells from PTSD patients than in cells from TC using both area under curve ($p<0.01$) and maximum inhibition of T-cel proliferation (DEX1000) ($p=0.02$) as readout. Medication was no significant covariate. The incidence of dexamethasone resistance was also significantly higher in PTSD patients than in TC. However, data from GR binding studies revealed that there were no significant differences in bindingaffinity Kd and Bmax between both groups.
Discussion: In concordance with other studies our sample of deployment-related PTSD is characterized by high scores of fatigue. Our findings in this population are suggestive for a decreased sensitivity of PBMCs to glucocorticoids in PTSD. These changes cannot be attributed to alterations in GR density or affinity but may be mediated by other intracellular processes downstream of GR activity.

90. Evidence That SSRI Response in Depression Is Influenced by a Functional Haplotype in *HTR1B* and the C-1019G SNP in *HTR1A*

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Sponsor: Travel Awardee, NIMH, 2005

Background: Selective serotonin reuptake inhibitors (SSRIs) are among the most commonly prescribed antidepressants. Although serotonin transporter (5HTT) is the main target of SSRIs, the delay in onset of its therapeutic effect strongly suggests that other genes in the serotonin and other neurotransmitter systems may be involved. A progressive desensitization of serotonin 1A (HTR1A) and 1B (HTR1B) autoreceptors has been shown to be associated with the delay in the onset of action of SSRIs and maybe with the therapeutic response.
Methods: We evaluated genetic variants in *HTR1A* and *HTR1B* genes to further assess their role in citalopram response in depressed patients. Three SNPs (T-261G, A-161T, Val287Val) in the *HTR1B* and one promoter SNP (C-1019G) in *HTR1A* were evaluated in 153 depressed patients treated with citalopram and followed-up for a period

of 12 weeks. A 16-Item Quick Inventory of Depressive Symptomatology Clinician rating (QIDS-C) was used to assess severity of symptoms. Differences in the QIDS scores at baseline and at week 12 among genotypes were evaluated by the t-test for independent samples. To evaluate the response over time a random coefficient model was fitted to the data using the linear mixed models (LMM) procedure in SPSS.
Results: Subjects homozygous for the -1019 G allele in HTR1A showed significantly higher baseline QIDS scores than those with the other genotypes ($p = 0.033$), and by 12 weeks had an even larger difference in final response rate ($p = 0.005$). Estimated haplotypes of 3 SNPs in *HTR1B* were classified according to previously reported *in-vitro* expression levels. Those homozygous for the high-expression level haplotype showed significantly slower response to citalopram ($p = 0.034$).
Discussion: Our results suggest that subjects with an enhanced capacity of HTR1B transcriptional activity are likely to respond less to SSRIs. Furthermore, we corroborate previous findings that the -1019G allele in *HTR1A* is involved in the severity of depression and poor antidepressant response outcome. The G variant displays differential binding efficiency of the repressors/enhancer-type transcriptional regulator (NUDR/DEAF-1). The HTR1A is repressed by NUDR/DEAF-1 in raphe cells at the C-, but not at the G-allele of the C(-1019)G SNP. The increase in concentration of 5-HT in the extracellular space induced by SSRIs, is offset by a negative feedback mediated by HTR1A and HTR1B autoreceptors, an effect that is attenuated after long-term treatment with SSRIs, likely due to desensitization of the autoreceptors. Desensitization of these receptors by SSRIs may be impaired by the presence of the G allele in HTR1A and the haplotype in HTR1B with an enhanced transcriptional activity.

91. The Mu-Opioid Receptor Polymorphism A118G Predicts Cortisol Responses to Naloxone and Stress

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Sponsor: Henry Kranzler

Background: Activation of the hypothalamic-pituitary-adrenal (HPA) axis is an important adaptive mechanism that enables the human body to return to homeostasis in response to physiological and psychosocial stressors. Cortisol, which is an end product of this activation, affects almost all physiological processes. HPA axis dysregulation has been shown to contribute to a number of neuropsychiatric and metabolic abnormalities. The magnitude of HPA axis activation is regulated by the interaction of environmental and genetic determinants. Estimations of the heritability of cortisol responses to psychosocial stress range from approximately 0.33 to 0.97. Moderate to high heritability has motivated a search for genes governing HPA axis dynamics. A candidate in this regard is the mu-opioid receptor (MOR). A polymorphism in the MOR (A118G) has been shown to increase beta-endorphin binding affinity, theoretically placing greater inhibitory tone on hypothalamic corticotropin-releasing hormone (CRH) neurons. We hypothesized that the minor allele (G) would predict cortisol responses to both pharmacologic (Naloxone) and physiologic (stress) activation of the hypothalamic-pituitary-adrenal (HPA) axis.
Methods: Healthy subjects (mean age 25.2 y, SD 9.2 y) completed a Naloxone challenge ($n = 74$) and/or the modified Trier Social Stress Test (TSST) ($n = 86$). For the Naloxone challenge, two baseline blood samples were obtained. Then, five increasing doses of IV Naloxone were administered at 30-min intervals and twelve additional blood samples were collected at 15-min intervals. The TSST consisted of 5-min public speaking and mental arithmetic exercises. Three baseline and 5 post-TSST blood samples were drawn.
Results: There were no statistically significant differences between genotype groups in terms of sex, race, smoking status, age, BMI, or level of education. The Naloxone challenge subjects as a group had significant ACTH and cortisol responses to Naloxone ($P < 0.001$ for each). ACTH and cortisol did not differ at baseline or following placebo by genotype. Compared to those homozygous for the A allele, subjects with the G allele had no significant difference in ACTH response, but did have a significantly greater cortisol response ($P = 0.046$) to Naloxone. Similarly, ACTH response by AUC analysis was not different by genotype, but subjects carrying the G allele had a significantly greater AUC cortisol response ($P = 0.041$) The TSST subjects as a group had significant ACTH and cortisol responses to the TSST ($P < 0.001$ for each). Compared to those homozygous for the A allele, subjects with the G allele had no significant difference in ACTH response, but did have a significantly lower cortisol response ($P = 0.044$) to the TSST. ACTH response did not differ between genotype group by AUC analysis. The area under the cortisol response curve for the G-allele group was less than that for the group homozygous for the A-allele with marginal statistical significance ($P = 0.058$).
Discussion: The minor allele (G) was associated with a robust cortisol response to Naloxone blockade, but a blunted response to psychosocial stress. We speculate that increased opioid avidity of the minor allele receptor contributes to the differential response to Naloxone versus stress.

92. 5HTTLPR Genotypes and Environmental Stress Interact to Affect Sleep Quality

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Background: Prior research has documented the involvement of serotonin (5HT) in sleep regulation. The 5HT transporter (5HTT) is an important regulator of 5HT function. A common 44-base pair deletion (s allele) polymorphism in the 5HTT gene-linked polymorphic region (5HTTLPR) is associated with reduced 5HTT transcription efficiency and reduced 5HT uptake in vitro. In this study we tested the hypothesis that 5HTTLPR genotype will be associated with sleep quality, both as a main effect and as moderated by a major life stressor. We defined major life stressor as being a caregiver for a close relative with Alzheimer's Disease (AD) or other major dementia, as we have shown that this stress does indeed impair sleep quality (Health Psychology, in press).
Methods: Subjects were 155 adults [118 (76%) Caucasian); 115 (74%) Female] who are primary caregivers for a spouse or parent with AD or other major dementia and 127 controls [97 (76%) Caucasian; 99(78%) Female] similar in age, sex and socioeconomic status without caregiving responsibilities. 5HTTLPR genotypes were assessed using the standard PCR method described by Lesch et al. (1996). Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989), with higher scores indicating poorer sleep quality. Multiple linear regression was used to examine whether 5HTTLPR genotype is associated, either as a main effect or as moderated by caregiver status, with sleep quality. Effects of race and gender were also evaluated. Initial analyses showed that s/s and s/l genotypes did not differ from each other in sleep quality but were both different from the l/l genotype; in the remaining analyses, therefore, we compared those subjects with the s/s or s/l genotype with those having the l/l genotype
Results: Caregivers had poorer sleep quality ($P<0.0001$) than controls, but 5HTTLPR genotype was not related ($P>0.83$) to sleep quality as a main effect. However, there was a significant group X 5HTTLPR interaction ($P=0.0065$), such that caregiver s allele carriers had poorer ($P<0.05$) sleep quality (mean PSQI of 11.16+/-0.42, SEM) than caregivers with the l/l genotype (10.00+/-0.49). Among controls the effect of the s allele was opposite, with s carriers having better

(<0.06) sleep quality (PSQI of 8.33+/-0.45) than controls with the l/l genotype (9.58+/-0.56). This group X 5HTTLPR effect on sleep quality was not moderated by race (P=0.76), but there was a significant (P=0.02) group X 5HTTLPR X gender interaction, such that the association of the s allele with poorer sleep quality in caregivers and better sleep quality in controls was present only in the female subjects; in males 5HTTLPR genotype was not associated with sleep quality in either caregivers (P=0.81) or controls (P=0.28).
Discussion: We found that 5HTTLPR genotype alone is not associated with sleep quality as measured by the PSQI. It does interact with an environmental stressor to affect sleep quality, but in opposite directions in caregivers compared to controls, and only in women, though there may have been limited power to find an effect in males. Among the women caregivers, those carrying the s allele report poorer sleep quality than those with the l/l genotype; in controls, s carriers report better sleep quality. Because the PSQI is valid measure of sleep quality [Carpenter & Andrykowski, 1998; Buysse et al., 1989], these findings suggest that women s allele carriers with caregiving responsibilities will be at higher risk of suffering problems associated with poor sleep quality than those with the l/l genotype. These findings also provide evidence for the recently highlighted (Moffitt, Caspi, & Rutter, 2005) importance of considering environmental exposures before concluding that there is no genetic effect on important phenotypes.

93. Evidence of Increased CRH Pulsatility in Major Depression

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Background: Increased activation of the HPA axis is a well described feature of major depression. ACTH and cortisol are secreted in pulses which are believed secondary to pulses of CRH. While increased levels of ACTH may indicate increased CRH activity or might indicate greater ACTH stores at the pituitary, an increase in the number of PULSES would be indicative of alterations in central ultradian rhythm generators driving the HPA axis. Such an increase would clearly point to abnormalities in brain areas controlling the circadian/ultradian rhythms of the HPA axis.
Methods: Evaluation of every 10 minute blood samples for ACTH following treatment with metyrapone every 4 hours for a total of 24 H in patients with major depression and normal control subjects. Samples were assayed with Immulite chemiluminescent assay for ACTH. Data were analyzed for pulses with Deconvolution.
Results: Preliminary data (8 pairs of MDD and age and sex matched normal controls) show a significant increase in the number of pulses of ACTH (19.8 vs 16.5 per 24H) as well as trends towards greater secretory amplitude (18.5 vs 13.9 pM) and shorter interpulse intervals 43.5 vs 41.4 min) Cortisol levels remained low throughout the 24 H (mean cortisol was 2.2 0.5 mcg/dl) and this was non-pulsatile in nature.
Discussion: These data are consistent with our 24 H studies of basal secretion (not under metyrapone) which found 26 vs 22 pulses in depressed women. Furthermore these studies suggest an increase in central elements driving the HPA axis in major depression. Increased number of pulses has been found in animal studies exploring inflammatory activation of the axis. These studies suggest alterations in the HPA axis "pulse generator" in depression, likely linked to alterations in other circadian and ultradian rhythms in major depression.

94. Identifying the Most Sensitive Measures and Definitions of Outcome for Pediatric Bipolar Disorder

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Background: Investigations of acute treatments for pediatric bipolar disorder have used different outcome measures and different definitions of response (e.g., 33% symptom reduction, 50% symptom reduction, or complex combinations of mood changes)(e.g., Frazier et al., 2001; Kafantaris, 1995; Kowatch et al., 2000; Wagner et al., 2002). This not only hinders comparison of findings across studies, but also makes it impossible to determine which measures are most sensitive to treatment response. Objective: To compare multiple outcome measures using the same set of definitions of treatment response in a single sample of youths receiving acute treatment for bipolar disorder. Measures were compared in terms of sensitivity to treatment effects at both the group level (using effect size measures such as Cohens d) and individual outcome using the Jacobson & Truax (1991) model popular in evaluating nonpharmacological treatments.
Methods: Secondary analysis of acute, open-label combination therapy (Li & DVPX) for patients ages 5-17 years diagnosed with BP I or BP II (N=87; Findling et al., 2003; Findling et al., 2005). Analyses included t-tests and Cohens d effect size to evaluate group treatment effects, as well as multiple definitions of individual response, including: (a) >=33% reduction in symptoms; (b) >=50% reduction; (c) 95% reliable change (per J&T, 1991); (d) reliable change plus moving more than 2 SDs away from the average for bipolar cases (J&T Definition A); (e) reliable change plus moving within 2 SDs of the nonclinical mean (J&T Definition B); (f) reliable change plus moving closer to the nonclinical than the bipolar mean (J&T Definition C); or (g) a composite definition including YMRS < 12.5, CDRS-R <=40, and CGAS >= 51 (per Findling et al., 2003). Correlations and kappas quantified associations between these definitions and expert clinician ratings on the CGI-Bipolar scale.
Results: All measures showed significant improvement, $p < .05$, with effect sizes ranging from d=.63 (self-reported depression on the GBI) to 1.64 (parent report of Biphasic/Hypomanic symptoms on the P-GBI). However, group results obscured considerable variability in individual outcomes: 8-14% of cases got worse over the course of the trial, depending on outcome. Response rates varied from 83% to 21% depending on outcome definition. Jacobson & Truax definitions were more conservative than the more widely used definitions. A total of 51 cases (59%) earned CGI-BP global improvement scores of 1 or 2, and 71% of cases met the composite definition of response. Clinician ratings on the YMRS and CGAS showed the best validity in terms of predicting CGI-BP change scores (median r of .59 for both measures), followed by the P-GBI depression (median r = .41), P-GBI hypomanic/biphasic (median r = .35), CDRS-R (r = .27), and then the adolescent GBI (median r = -.11 and .20, both $p > .05$).
Discussion: All instruments were sensitive to treatment effects using conventional group-level test statistics, with medium to extremely large effect sizes. However, group data masked wide variation in individual outcome, including that several patients worsened. There were also marked differences in response rate due to choices of operational definition and measure. Teen self report showed the smallest treatment effects and poorest agreement with clinician ratings. Results suggest that clinician ratings deserve continued prominence in evaluating outcomes. Parent report on the P-GBI may prove useful, too, particularly in situations where clinicians have variable training and/or different benchmarks (e.g., multi-site trials) or limited experience (e.g., non-research settings) with rating manic or depressive symptoms.

95. Robust, Rapid and Relatively Sustained Antidepressant Effects with a Single-Dose of an NMDA Antagonist in Treatment-Resistant Major Depression: A Double-Blind Placebo-Controlled Study

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Sponsor: Mauricio Tohen

Background: Although there are many effective treatments for major depression, existing therapies have a lag of onset of action of several

weeks, resulting in considerable morbidity and high risk of suicidal behavior especially in the first 9 days of starting antidepressants. Exploring pharmacological strategies that have rapid onset of antidepressant effects within hours or a few days, and — that are sustained — would have an enormous impact on patient care. Converging lines of evidence suggest the role of the glutamatergic system in the pathophysiology and treatment of mood disorders. A previous study suggested that a single intravenous dose of the non-competitive NMDA antagonist ketamine produced a rapid but transient antidepressant effect in 7 subjects with treatment-resistant depression. Together, these data suggests that the glutamatergic system may play a role in the pathophysiology and treatment of depression and those agents which directly modulate glutamatergic neurotransmission may represent a novel mechanism for effective and rapid onset of antidepressants. The objective of this study was to determine whether a rapid antidepressant effect can be achieved with an NMDA antagonist in subjects with treatment-resistant major depression.
Methods: After a 2-week drug-free period, 17 subjects with DSM-IV major depression, (treatment-resistant [failed a least 2 antidepressant trials]), were given an intravenous infusion of either ketamine hydrochloride (0.5 mg/kg) or placebo (saline solution) on 2 test days, a week apart, in a randomized, double-blind, cross-over study. 16 subjects received ketamine and 12 placebo, 5 subjects did not receive placebo after ketamine infusion as they maintained a response for more than 7 days and 1 subject was discontinued from the study for medical reasons after a placebo infusion. Subjects were rated on the Hamilton Depression Rating Scale (HDRS-17) just prior to the infusion and at +0, +40, +80, +110, +230 minutes, days 1, 2, 3 & 7 upon completion of the infusions.
Results: Using a linear mixed model, subjects on ketamine showed significant improvement in depression compared to placebo within 80 minutes after injection that remained significant throughout the following week. The effect size for the drug difference was very large (d=1.83, CI= 1.24-2.43, p=0.000) after 24 hours and large (d=.74, CI=0.14-1.34, p=0.015) after 1 week. Of the 16 subjects treated with ketamine, 15 (94%) met response (50% improvement on HDRS) and 11 (69%) met remission (≤7 HDRS) criteria at some point within the week. 5 subjects maintained response for at least 1 week, 2 of which maintained response for at least 2 weeks. By contrast, 1 of 12 (8%) subjects on placebo achieved a response only on the same day. Perceptual disturbances occurred in most subjects on ketamine which lasted the duration of the infusion. No serious adverse events occurred.
Discussion: Robust antidepressant effects with a single intravenous dose of an NMDA antagonist were obtained within 80 minutes and continued to remain significant for 1 week. Overall, the results of this study are consonant with hypotheses of NMDA receptor dysfunction in depression. Further study to discern whether these acute effects could ultimately be sustained for therapeutic purposes appears warranted.

96. Responsiveness of Mice Deficient in Beta Adrenergic Receptors to Desipramine and Beta Adrenergic Agonists: Behavioral Effects Observed Using the Forced-Swim Test (FST) and Differential-Reinforcement-of-Low-Rate (DRL) Schedule
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Sponsor: James O'Donnell

Background: Beta adrenergic receptors have been shown to be involved in antidepressant activity, but the relative roles of the beta-1 and beta-2 subtypes have not been fully elucidated.
Methods: Antidepressant- or depressive-like effects were determined in mice under a differential-reinforcement-of-low-rate (DRL) 36-sec schedule and in the forced-swim test (FST). Cyclic AMP concentrations also were determined in vitro in slices of the cerebral cortex from different genotypes of mice.
Results: Mice deficient in beta-1 or both beta-1 and beta-2, but not beta-2, adrenergic receptors (beta-1 -/-, beta-1/2 -/-, and beta-2 -/-, respectively) displayed depressive-like effects, as evidenced by increased immobility compared to the wild-type (WT) control in the FST. Administration of dobutamine (Dob; 30 mg/kg) or clenbuterol (Clen; 0.5 mg/kg), selective beta-1 and beta-2 adrenergic agonists, respectively, or desipramine (Des; 20 mg/kg), decreased immobility in the WT mice. However, the effects of Dob and Clen were absent in beta-1 -/- and beta-2 -/- mice, respectively, or beta-1/2 -/- mice. The effect of Des was not altered in any of the gene knockout mice. In the WT or gene knockout mice under a DRL 36-sec schedule, Des (10-30 mg/kg) produced antidepressant-like effects, i.e. decreased response rates and increased reinforcement rates, despite the different baselines observed in the lines. Dob (10-30 mg/kg) was effective in WT and beta-2 -/- mice; Clen (1-10 mg/kg) was effective in WT and beta-1 -/- mice. Neither of the agonists produced any significant effects on DRL behavior in beta-1/2 -/- mice. In slices of cerebral cortex from WT mice, incubation with isoproterenol (0.01 and 0.1 uM), a non-selective beta adrenergic agonist, Dob (1 and 10 uM), or Clen (0.1 and 1 uM) increased cyclic AMP concentrations in the presence of the PDE4 inhibitor rolipram (1 uM); these were blocked in cortical slices from beta-1 -/- and beta-1/2 -/-, but not beta-2 -/- mice.
Discussion: While beta-1 and beta-2 adrenergic receptors were involved in antidepressant-like effects of dobutamine and celnbuterol, respectively, neither is critical for the antidepressant-like effect of desipramine. These were consistent with the changes in cAMP concentrations in slices of the cerebral cortex from different genotypes of mice. These results suggest that while beta-1 adrenergic receptors play an important role in the mediation of antidepressant activity, the behavioral effects of desipramine are mediated, at least in part, by a different mechanism (Supported by research grants from NIMH).

97. Lithium Regulates P2X7 Receptor Expression and ATP Activation in Astrocytes: A Novel Therapeutic Target in the Treatment of Bipolar Disorder?
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Sponsor: James Barrett

Background: Mood disorders are associated with a reduction in regional CNS volume and cell atrophy or neuronal and glial loss. Lithium, a mainstay in the treatment of mood disorders, is known to robustly enhance neurogenesis and increase the expression of glial fibrillary acidic protein (GFAP) in rodent hippocampus, suggesting an important role for lithium in the regulation of glial cell growth and functions. Recent evidence suggested that ATP acting via ionotropic (P2X) purinergic receptors might be involved in signaling between glial cells and within glial-neuronal networks. The P2X7 receptor, known as the cytolytic P2Z receptor, has been implicated in signalling between neuron and astrocytes, and has recently been postulated to represent a candidate gene for recurrent mood disorders. P2X7 receptors have been proposed as mediators of inflammation, and a potential role in neurodegeneration has been suggested. The P2X7 receptor shares 35-40% homology with other P2X receptors. It has two hydrophobic membrane-spanning domains and an extracellular loop, and forms transmembrane ion channels. Under normal conditions, extracellular nucleotides are present in only low concentrations. However, activated immune cells, such as lymphocytes, macrophages, microgli, and platelets, and dying cells may release high concentrations of different nucleotide di- and tri-phosphates into the extracellular space. Under inflammatory conditions, P2X7 receptor activation stimulates the induction of multiple cytokine pathways that may co-ordinate inflammatory responses, and triggers

massive transmembrane ion fluxes (particularly influx of Ca2+ and Na+, and efflux of K+) and the formation of non-selective plasma membrane pores that result in cell death. In contrast to their neuronal counterpart, the function of P2X receptors in CNS glial cells is largely unknown.
Methods: By Westen blot protein analysis, immunocytochemistry and immunohistochemistry, we examined expression of P2X7 receptors in astrocytes in vivo and in vitro and examined effect of lithium (Li) on the expression of P2X7 in astrocytes.
Results: We found that P2X7 receptor expressed in astrocytes. P2X7 positive cells can be GFAP- and S100beta- positive, suggesting a colocolized of astrocyte proteins and P2X7 receptor in CNS. Moreover, we also found that Li (0.5-1.0 mM) resulted in significantly decreased expression of P2X7 receptor in cultured astrocytes. Finally, we also found chronic (5-day) Li-treatment significantly blocked ATP-induced influx of Ca2+ in astrocte.
Discussion: Our data demonstrated that P2X7 expresses in astrocyte, and its expression levels can be regulated by chronic treatment of ithium at therapeutically relavent concentrations. Considering P2X7 receptor's role in ATP's regulation on Ca2+, this may be the molecualr mechanism that ATP-induced influx of Ca2+ can be blocked by lithium in astrocyte. Our data provide evidence showing that P2X7 receptor in astrocytes may be a therapeutic target for mood disorder treatments.

98. Abnormal Expression of Transcripts for GKAP and Shank Suggest Alterations of Interactions Between Metabotropic and Ionotropic Glutamate Receptors in Schizophrenic Brain

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Background: Abnormal expression of molecules involved in glutamatergic signaling has been found in schizophrenia. While much research has focused on changes of the ionotropic NMDA and AMPA receptors (iGluRs) and their associated post-synaptic proteins in psychiatric illness, changes of the metabotropic G-protein coupled glutamate receptors (mGluRs) and their associated intracellular proteins have received less attention. The family of mGluR glutamate receptors encompasses eight different receptors, which based on functional and structural characteristics have been divided into 3 subgroups. The mGluR1 and mGluR5 receptors, which constitute the group I mGluRs, are expressed in both neurons and glia. In excitatory synapses mGluR1/5 are predominantly located in the periphery of the post-synaptic density (PSD) where they are linked to NMDA and AMPA receptors through a dense intracellular network of anchoring proteins. Emerging evidence suggests that these anchoring molecules, which include Homer, Shank and GKAP, serve functions not exclusively as structural units of the PSD, but also as dynamically interacting proteins involved in synaptic development and remodeling, thus providing a connection between these synaptic events and coordinated iGluR and mGluR function. Previous studies in postmortem brains have identified abnormal cortical and subcortical expression of both iGluRs and mGluRs in schizophrenia.
Methods: In this work, we have measured expression of transcripts for GKAP and of the Shank isoforms 1 and 3 in dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) in postmortem brains from elderly schizophrenic patients and comparison subjects.
Results: While we found no changes of GKAP transcript expression in schizophrenia in either DLPFC or ACC, expression of Shank1 in ACC and Shank3 in DLPFC was increased.
Discussion: Since Shank, with Homer, is an important mediator of mGluR associated regulation of intracellular Ca2+ release, and is a required binding partner for GKAP and Homer in bridging iGluRs and mGluRs, its abnormal expression might compromise normal cell biological processes in the glutamatergic synapse in schizophrenia. This work was supported by MH53327 and by the Stanley Foundation.

99. Correlations Between REM Sleep EEG Spectral Analysis and CSF GABA in Clinically Stable Drug-Free Patients with Schizophrenia: A Pilot Study

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Background: GABA-related biomarkers have been shown to relate to structural, clinical and REM sleep disturbances in schizophrenia (van Kammen et al., 1994; 1998). GABA activity has also been shown to modulate REM sleep activation (Boissard et al., 2003) as well as beta1 EEG spectral power during sleep (Bastien et al., 2003; Feinberg et al., 2000). The present study aims at evaluating the relationship between CSF GABA markers and REM sleep EEG beta1 spectral power in clinically stable drug-free patients with schizophrenia.
Methods: Ten drug-free and clinically stable male inpatients (age: 39.4 +/- 4.4, range: 25 - 49 years) diagnosed with schizophrenia (DSM-III-R, APA 1987), participated in the present study. Sleep recording methods have already been described in Nofzinger et al. (1993). All participants sleep three nights in a sleep laboratory after six weeks of neuroleptic withdrawal. The third night was used for the present analysis. Sleep stages were visually identified using 60-sec epochs in accordance with Rechtschaffen and Kales (1968). Spectral analysis was performed on the C3 EEG channel. For each 60-sec epoch, absolute and relative EEG spectral power were extracted using FFT for delta (0.75-3.75 Hz), theta (4.00-7.75 Hz), alpha (8.00-12.75 Hz), beta1 (13.00-19.75 Hz) and beta2 (20.00-30.00 Hz) frequency bands. Thirty epochs without artifact were proportionally selected from the three first REM sleep periods. The mean spectral power for each frequency band was calculated. The lumbar puncture and assay methods have been described in van Kammen et al. (1998). Lumbar punctures were obtained with the patient in the lateral decubitus position. Twelve ml of CSF was collected, mixed, put on ice and stored at -80 degrees Celcius. CSF GABA was measured using high performance liquid chromatography with post-column derivation and fluorescence detection. Values of CSF GABA were adjusted for storage time using linear regression. Correlations between CSF GABA concentration levels and beta1 spectral power were performed using Spearman Rho. Correlations with other frequency bands were done as an exploratory analysis.
Results: Two patients presented too many artefacts on spectral analysis and have been removed from the analysis. A positive correlation was observed between CSF GABA concentration level and REM sleep EEG absolute beta1 spectral power ($r=.78$, $p<.02$). A trend to a positive correlation with absolute alpha spectral power was observed ($r=.66$, $p<.07$).
Discussion: The present results show that high GABA activity is related to high beta1 EEG spectral power during REM sleep in drug-free and clinically stable patients with schizophrenia. This observation corroborates previous studies showing that benzodiazepines increase beta1 EEG activity during sleep (Feinberg et al., 2000). Previous results in never treated patients with schizophrenia have shown that Increased REM sleep EEG Beta1 spectral amplitude was related to increased negative symptoms but decreased positive symptoms (Poulin et al., 2001). Thus, increased REM sleep EEG Beta1 spectral power may reflect a relatively increased GABA activity in those patients with schizophrenia who presents with a higher negative/positive symptoms ratio.

100. Medial Temporal Lobe Structures and *RGS4* Polymorphisms in First-Episode Schizophrenia

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Sponsor: Past Travel Awardee, PMRTP, 2004

Background: Medial temporal lobe (MTL) structures (hippocampus, parahippocampal gyrus, amygdala and entorhinal cortex) have been consistently implicated in the pathophysiology of schizophrenia. These regions contain a rich network of monoaminergic synapses where neurotransmission is mediated through G-protein coupled receptors. Allelic variants in the gene that encodes the regulator of G-protein signaling subtype 4 (*RGS4*) has been associated with schizophrenia in seven independently ascertained populations. RGS4 negatively regulates G-protein activation. Prior postmortem studies have reported underexpression of *RGS4* in the dorsolateral prefrontal cortex (DLPFC) and we reported an association between *RGS4* genotypes and DLPFC volume in first-episode schizophrenia patients. *RGS4* is differentially expressed in the MTL structures; parahippocampal gyrus (PHG) moderately expresses *RGS4* whereas hippocampus expresses minimally. Based on these findings, we hypothesized that the parahippocampal gyrus (PHG) will show volumetric changes across the *RGS4* genotypes whereas hippocampus will not.
Methods: We recruited a series of first-episode schizophrenia patients (n=24) and matched healthy control subjects (n=29) and acquired structural MRI scans on all these subjects. We genotyped these subjects using single base extension reaction assay and measured the MTL structures (PHG, hippocampus, entorhinal cortex and amygdala) using the methods that were published earlier.
Results: Using MANOVA, we found a significant diagnosis x genotype interaction for SNP4 and 18 for the MTL structures (Roy's Largest Root, F=2.32, p=0.036). Within group MANCOVA using age and gender as covariates revealed that the PHG volume was significantly different as a function of SNP4 (Model p=0.05; right PHG, F=5.58, p=0.012; left PHG, F=2.39, p=0.1) and SNP18 (Model p=0.054; right PHG F(2,24)=5.43, p=0.014; left PHG, F(2,24)=2.32, p=0.12) alleles within patients but not in controls. Patients homozygous for allele T (n=11) had smaller volumes compared to those homozygous for allele G (n=4) (Posthoc Bonferroni tests, right PHG p=0.008; left PHG=0.07; Fig 1). We did not find such differences in the volumes of hippocampus, entorhinal cortex, amygdala or total brain volume.
Discussion: Our main finding is that among the MTL structures parahippocampal gyrus showed volumetric differences within patients across the *RGS4* genotypes but not control subjects suggesting an interaction with other disease related factors. These results suggest that the volumetric changes associated with *RGS4* polymorphisms may be regionally specific, possibly reflecting the pattern of expression of *RGS4*. These observations are consistent with our earlier report of the association with dorsolateral prefrontal cortex volume with *RGS4* genotypes. Furthermore, we have reported earlier that the PHG volume negatively correlated with severity of delusions and thought disorder scores. Further analyses are being carried out to examine the association of *RGS4* polymorphisms with these clinical features.

101. The Consortium on the Genetics of Schizophrenia (COGS): Reliability and Validity of Standardized Assessement of Basic Oculomotor Function in Schizophrenia Patients, First Degree Relatives and Controls in a Multisite Study

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Sponsor: Elaine Peskind

Background: The COGS is an ongoing NIMH funded 7-site collaboration investigating the genetics of schizophrenia endophenotypes. Documentation of the feasibility and reliability of obtaining oculomotor data as part of a larger research battery, administered at multiple sites, is crucial if these tasks will be used to assess endophenotypes in schizophrenia. Significant schizophrenia-control differences in smooth pursuit performance have been well documented in single site studies. We report on the effectiveness of cross-site training, standardization of methods and equipment, as well as the initial data analysis of smooth pursuit and prosaccade measures.
Methods: Two identical smooth pursuit and one prosaccade task were administered as part of a battery of tests quantifying six endophenotypes in schizophrenia patients, their families, and normal controls. Training consisted of an extensive, in-person training session, conference calls, and review of a detailed video. Ongoing quality control consisted of biweekly phone calls, site visits and reviews of methods. Data from all 7 sites were transmitted to a central data analysis site. Blinded data was first reviewed visually; data with unacceptably poor quality were discarded. Primary prosaccades, smooth pursuit artifacts, smooth pursuit eye movement and saccade subtypes were identified and characterized with a computerized pattern recognition algorithm.
Results: We have collected oculomotor data from 512 subjects thus far. Data were acceptable for 98 probands, 214 first-degree relatives, and 135 controls. Prosaccade and smooth pursuit data quality were unacceptable for 59 and 57 subjects, respectively and 20% of all smooth pursuit tracking was designated unusable by the computer algorithm. Latency and amplitude of prosaccades did not vary among the three subject groups. Smooth pursuit gain was significantly lower ($p<0.001$), and catch-up and intrusive saccade frequency was significantly higher ($p<0.05$) among probands, but these variables did not differ between first-degree relatives and controls. The smooth pursuit gain of schizophrenia patients was lower than the other two groups at 6 of the 7 sites. Correlations between the gain in the two smooth pursuit tasks, calculated for each site individually, were highly significant at all sites.
Discussion: After extensive training, laboratories at 7 sites, some with no prior experience in oculomotor research, collected high quality prosaccade and smooth pursuit data. Proband-control differences were consistent with those reported in prior single site studies. High correlation between gain during the two smooth pursuit tasks, and lack of major differences in the patterns of data from the sites, indicates that training and quality assurance efforts were successful.

102. Disrupted Integration of Prefrontal and Hippocampal Activation in Schizophrenia During Word Encoding and Recognition

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Background: Functional imaging studies of episodic memory often find reciprocal abnormalities in the prefrontal versus temporal limbic brain function of individuals with schizophrenia. That is, reduced frontal lobe function is accompanied by overactivation in hippocampal regions, and prefrontal hyperactivity is accompanied by reduced hippocampal response. These reciprocal abnormalities have provided support to the hypothesis that schizophrenia may disrupt the normal functional connectivity of prefrontal and temporal limbic brain structures (Friston & Frith, 1995). The current study aims to directly test this disrupted frontotemporal connectivity hypothesis.
Methods: BOLD fMRI data were examined for a sample of 13 patients with schizophrenia and 13 healthy controls who performed a levels-of-processing word encoding and recognition paradigm (Ragland et al., 2003). Previous whole brain SPM2 analysis of these data (Ragland et al., in press) revealed that patients and controls activated ventrolateral prefrontal cortex with no group differences in prefrontal function. However, patients showed overactivation in the parahippocampal gyrus. The current study used a region-of-interest

(ROI) analysis approach to examine relationships between these two brain regions. ROIs were constructed based on demarcation of Brodmann areas (BA) within Talairach space. The ventrolateral prefrontal cortex (VLPFC) included all voxels belonging to BA47, and the hippocampal formation (HIP) included voxels belonging to the hippocampus and parahippocampal gyrus. These structural ROI masks were applied to the SPM{z} maps of each individual and thresholded at $p<.05$, uncorrected. SPM{z} maps were based on correctly performed trials only, and the liberal threshold insured an adequate number of voxels for calculation of a stable mean value, without including voxels unrelated to task performance. Resulting mean percent signal change values (%Signal) were calculated within each hemisphere, separately for word encoding and recognition conditions.
Results: There were no group differences in %Signal in VLPFC or HIP regions for either encoding or recognition conditions, with both groups demonstrating a 0.4 to 0.5 % increase in signal. Examination of Spearman correlations during encoding did not reveal any significant relationships in the right hemisphere for either group. However, controls had a significant correlation between left hemisphere VLPFC and HIP activity ($r=.78$, $p<.05$) that was not present in the patient sample ($r=.20$, ns). A similar pattern was seen for the right hemisphere during word recognition. Healthy subjects demonstrated a positive correlation between VLPFC and HIP %Signal ($r=.65$, $p<.05$), that was not present in the patient sample ($r=.52$, ns).
Discussion: These results provide additional support for a model of disrupted connectivity between prefrontal and temporal limbic brain regions in schizophrenia. Although there were no group differences in percent signal change for either region, only controls showed appropriately lateralized coupling between the prefrontal cortex and hippocampal formation during correct word encoding and recognition.

103. Bilateral Reduction of Caudate Volumes in the Young, Asymptomatic, Genetically at Risk Offspring of Patients with Schizophrenia

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Sponsor: Gerald Sarwer-Foner

Background: The basal ganglia represent target fields for dopaminergic tracts that are believed to be involved the pathophysiology of schizophrenia. Basal ganglia play a critical role in higher cognitive functions such as attention, working memory, and goal-directed behavior. The increased caudate volumes seen in chronic schizophrenia may be related to antipsychotic treatments; drug naive patients have smaller caudate volumes suggesting an illness related striatal pathology. It is unknown whether this reflects a biomarker of susceptibility to the illness, or is a state dependent alteration of new onset illness. . Studying at-risk relatives can help clarify this question as the risk of schizophrenia increases by the proximity of genetic relationship. In this study we measured the caudate volume in adolescents with at least one parent with schizophrenia.
Methods: Right and left Caudate nucleus were measured in a semi-automated method using MRI (1.5 mm T1 coronal images resampled in AC PC Talairach space) in 52 young non-psychotic offspring (HR=29 males and 23 females, mean age 15.4 years, Std 4.37) of patients with schizophrenia and 53 normal comparison subjects similar in age and sex (NC=27 males and 26 females, mean age 16.5 years Std 4.37) with no family psychiatric history.
Results: Caudate volumes were significantly reduced in the HR subjects bilaterally (right NC = 3.58 cc, Std 0.5, HR=3.26 cc, Std 0.5, $p<0.0006$, and left NC=3.57 cc, Std 0.5, HR=3.26 cc Std 0.5, $p<0.001$. Intracranial volumes did not differ across the groups ($p=.53$).
Discussion: These findings provide new evidence that caudate volume reduction may be a trait related, premorbid neuroanatomical abnormality in the genetically vulnerable individuals. These observations may provide the neuroanatomical basis for the motor and neurocognitive abnormalities seen in HR subjects. Further studies and follow up will help understand the role of the striatum in the premorbid vulnerability and progression to later schizophrenia.

104. The Orexin-1 Receptor Antagonist SB-334867 Reverses the Depolarization Inactivation of A9 and A10 Dopamine Neurons Caused by Chronic Administration of Haloperidol and Olanzapine

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Sponsor: Barry Jacobs

Background: Chronic administration of antipsychotics leads to depolarization inactivation of dopamine neurons in the ventral tegmental area (A10) and substantia nigra pars compacta (A9). We have previously shown that the selective orexin-1 antagonist SB-334867 can block the acute excitatory effects of haloperidol and olanzapine on A9 and/or A10 cells. Thus, we examined the effects of SB-334867 on the effects of chronic administration of haloperidol or olanzapine on A9 and A10 cells.
Methods: We used single-unit, extracellular recordings to examine the number of spontaneously active A9 and A10 cells in chloral hydrate anesthetized male Sprague-Dawley rats. Osmotic minipumps were used for the chronic administration of compounds. The electrode was passed through 9 tracks in A9 or A10; each track was separated by 0.2 mm. After six tracks were made, SB-334867 was administered i.v. and three additional tracks were made.
Results: Acute administration of SB-334867 did not alter the number of spontaneously active A9 or A10 cells in animals receiving chronic vehicle, but did reverse the decrease in the number of spontaneously active A9 and/or A10 dopamine cells caused by the chronic administration of haloperidol (1 mg/kg/day X 21 days, sc) or olanzapine (10 mg/kg/day X 21 days, sc).
Discussion: These results indicate that activation of orexin-1 receptors plays an important role in the effects of antipsychotic drugs on dopamine neuronal activity and may play an important role in the clinical effects of antipsychotic drugs. This work was funded by Eli Lilly and Co. and carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and Eli Lilly and Co. Animal Care and Use Policies.

105. Second-Generation Antipsychotic Exposure and Patterns of Change in Metabolic Adverse Effects: A Longitudinal Pharmacoepidemiology Study from 1988 to 2002

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Sponsor: Larry Stein

Background: Individuals with schizophrenia are at higher risk for developing diabetes and cardiovascular disease. Factors such as unhealthy life styles and decreased access to health care contribute to a life expectancy that is 20% shorter in patients with schizophrenia than the general population. Additional modifiable risk factors include exposure to second generation antipsychotics (SGAs) that have been associated with metabolic syndrome. The precise quantitative relationship of SGAs to individual components of the metabolic syndrome (e.g. obesity and diabetes), however, has not been studied in a longitudinal naturalistic clinical setting. In an effort to delineate the relationship between the first introduction of SGAs in 1991 and the subsequent emergence of SGA-associated metabolic syndrome, a pharmacoepidemiology study was conducted using a large data base consisting of 5 to 8 million inpatient hospital stays per year sampled to approximate a 20-percent stratified sample of the US community hospitals from 1988 to 2002. The Nationwide Inpatient Sample (NIS,

Healthcare Cost and Utilization Project) is the only national hospital database with discharge information on all patients, regardless of payer, including persons covered by Medicare, Medicaid, private insurance, and the uninsured.
Methods: Hospital discharge data on inpatients with an ICD-9 diagnosis of schizophrenia, schizoaffective or psychotic disorder (hereafter referred to as cases) from 1988 to 2002 were obtained from the NIS. A control sample comprised of a random inpatient sample, created by exclusion of the cases, was selected in each study year. The net prevalence difference in obesity, diabetes, and surrogates for decompensated diabetes (diabetic ketoacidosis and hyperosmolar coma, DKA/HO) were calculated by subtracting the prevalence data in controls from that in cases in each year.
Results: Over the period of 1988 to 2002, the difference in prevalence of all end points in cases increased. For obesity, the prevalence of obesity in cases with a psychotic illness was 14.7% higher than controls in 2002. By contrast, in 1988 prior to the clinical adoption of SGAs, the obesity prevalence in cases was only 4.7% higher than controls. For diabetes, the prevalence was 1.5%-2.0% lower in cases compared to controls between 1988 to 1997. Beginning in 1998, however, a stepwise marked increase in net prevalence of diabetes was observed, reaching a prevalence that was 2.2% higher than controls by 2002. Similarly for DKA/HO, the prevalence was initially lower in cases between 1988 to 1992, equivalent from 1993 to 1998, and became marginally higher than controls from 1999 to 2002 (0.05%). Statistical trend analysis using regression of the difference in prevalence of each metabolic adverse event indicated a significant increase, as evidenced by positive slopes from 1988 to 2002 for obesity (slope = +0.98, $p<0.01$), diabetes (slope = +0.78, $p<0.01$) and DKA/HO (slope = +0.81, $p<0.01$). Using 1996 as an inflection point when adoption of SGAs reached 50% of the new prescriptions for antipsychotics, we observed a significantly higher prevalence difference for obesity in cases than controls during 1997-2002 (11.7% ± 1.9) (mean ± SD) compared to the 1988-1996 period (6.4% ± 1.6) ($p<0.001$).
Discussion: These longitudinal data provide a quantitative estimate of the marked increase in SGA-associated metabolic side effects in a large sample of inpatients drawn from US community hospitals. The increased prevalence of these side effect over the 15 year study period appears to parallel the rising market penetration of SGAs. Supported by: VISN 22 Mental Illness Research, Education, and Clinical Center (www.mirecc.org)

106. MEG Gamma Band Activity Reduced in Psychotic Adolescents

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Background: Gamma band (40Hz) activity has been implicated in cortical information transfer. Gamma band activity has been shown to be reduced in adults with schizophrenia. Further, the auditory sources of gamma band activity have been reported to have reduced asymmetry in psychotic patients. Gamma band activity in children or adolescents with psychosis, however, has not yet been reported.
Methods: We studied 25 subjects (7 with schizophreniform psychosis, 8 with affective psychosis, and 13 controls). MEG was recorded with a 37 channel Magnes I gradiometer system. Steady state fields were recorded in response to contralateral 500msec 40Hz pulse trains. The power spectrum of the steady state responses were analyzed using using wavelets, a time-frequency decomposition methodology . This analysis was applied both to the phase locked (evoked) and non-phased locked (induced) activity.
Results: Phase locked 40Hz power was significantly lower in the psychotic adolescent group compared to controls (p=.053). There were no apparent differences between the schizophreniform and affective psychoses, although the number of subjects in each group is too small to detect small effects.
Discussion: These findings are compatible with a disturbance in thalamocortical oscillatory mechanisms in adolescents with psychosis. The observation that phase locked power, and not induced power, shows a difference in psychotic adolescents may imply an inability to create and/or maintain a 40Hz oscillation, as opposed to any trial-to-trial variations during the course of the experiment.

107. Randomized Comparison of Olanzapine versus Risperidone for the Treatment of First Episode Schizophrenia: Four Month Outcomes

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Sponsor: Arthur Rifkin

Background: Studies with first episode populations offer the unique opportunity to examine the effectiveness and side effects of medications without the confounding effects of prior medication use. The Preventing Morbidity in First Episode Schizophrenia study compares outcomes with treatment with olanzapine and risperidone over three years in a variety of domains. This report presents response rates, negative symptom outcomes and side effects over the first four months of treatment.
Methods: One hundred twelve subjects (70% male; mean age 23.3 (SD = 5.1) years) with a first episode of schizophrenia, schizophreniform or schizoaffective disorder were randomly assigned to treatment with olanzapine (2.5 to 20 mg daily) or risperidone (1 to 6 mg daily). Response criteria are: a rating of 3 (mild) or less on the following SADS-C+PD items: severity of delusions; severity of hallucinations; impaired understandability; derailment; illogical thinking; bizarre behavior, and a rating of very much improved or much improved on the CGI improvement item. This level of improvement had to be maintained on two consecutive ratings.
Results: Response rates were 43.7% (95% CI: 28.8%, 58.6%) with olanzapine and 54.3% (95% CI: 39.9%, 68.7%) with risperidone (p=0.35). The percent of responding subjects having a subsequent rating not meeting response criteria was higher among subjects assigned to olanzapine (40.9%; 95% CI: 16.8%, 65.0%) than those taking risperidone (18.9%; 95% CI: 0%, 39.2%) but the difference was not statistically significant (p=0.08). There were no differences between the medications in negative symptom outcomes. Motor side effects: EPS scores were slightly higher with risperidone [1.3 (95% CI: 1.2, 1.5)] than with olanzapine [1.1 (95% CI: 0.9, 1.3)] but the difference was not statistically significant (p=0.06). The frequency of akathisia was low and did not differ between the medications. Weight gain: Weight gain was more with olanzapine than with risperidone (p=0.01). Percent gain from baseline weight at 16 weeks with olanzapine was 17.1% (95% CI: 13.8%, 20.4%) and 11.4% (95% CI: 8.4%, 14.6%) with risperidone.
Discussion: Both olanzapine and risperidone are effective at promoting response. There may be differences between the medications in stability of response and side effects.

108. Olanzapine and Clozapine Rapidly Decrease Whole-Body Insulin Sensitivity in Rats: Implications for the Diabetic Liability of Certain Antipsychotics

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Sponsor: B. Kenneth Koe

Background: To study underlying mechanisms of antipsychotic-induced hyperglycemia and diabetes,[1,2] we used acute models to examine properties that contribute to early metabolic effects of antipsychotics, i.e. before compensatory mechanisms or weight gain

confound the results. Recently we showed that olanzapine and clozapine inhibit cholinergic-stimulated insulin secretion, which could lead to hyperglycemic events,[3] while the present study investigated acute effects of antipsychotics on insulin sensitivity in vivo and on glucose transport in vitro.
Methods: *Insulin sensitivity.* Using a hyperinsulinemic, euglycemic clamp model (constant infusion with insulin 3 mU/kg*min and somatostatin 3 mg/kg*min) in 5 hr fasted conscious male Wistar rats, the Glucose Infusion Rate (GIR) to maintain euglycemia was monitored and used as an index of whole-body insulin sensitivity. Once steady state was achieved, drug or vehicle was administered sc and GIR was monitored for 120 min. *Glucose uptake in muscle.* Changes in basal and insulin-stimulated 2-deoxyglucose uptake were measured in the absence and presence of olanzapine in epitrochlearis muscle isolated from naive female CD rats, as well as in muscle isolated from rats that were treated 1 hr before with vehicle or 10 mg/kg sc olanzapine.
Results: *Insulin sensitivity.* Olanzapine (1, 3.2, & 10 mg/kg s.c.) and clozapine (1, 3.2, & 10 mg/kg sc) caused highly significant ($P<0.001$), dose-dependent reductions in GIR, starting within 40 min with maximal reductions from 53-68%. Ziprasidone (3.2, 10 or 32 mg/kg sc) and risperidone (2 mg/kg sc) had no effect on GIR, while all compounds showed dose-dependent sedative-like behavioral responses. *Glucose uptake.* Incubations with olanzapine (0.1- 10 μM) did not affect basal or insulin-stimulated 2-deoxyglucose transport in isolated epitrochlearis muscle from naive rats. Likewise, in muscle from rats treated with 10 mg/kg sc olanzapine, which resulted in olanzapine muscle levels of 7 μM, basal and insulin-stimulated glucose uptake was unaffected and subsequent incubations with olanzapine (0.1- 10 μM) had no effect.
Discussion: Olanzapine and clozapine, antipsychotics associated with an increased propensity for diabetes,[1,1,2] significantly and rapidly impair whole-body insulin action in rats after a single dose, prior to any effect on body weight. Since these compounds do not inhibit glucose transport in muscle, the decreased GIR is most likely a consequence of elevated hepatic glucose output. In contrast, high doses of antipsychotics with a limited (risperidone) or minimal (ziprasidone) diabetic risk had no effect. Since the euglycemic clamp experiments are carried out under hyperinsulinemic conditions, direct effects on insulin secretion do not play a role in olanzapine- or clozapine-induced decreases in insulin sensitivity in this paradigm. In addition to the direct impairment of cholinergic-stimulated insulin secretion from β-cells,[3] these effects could therefore represent an important mechanism that contributes to hyperglycemia or acute ketoacidosis reported in patients on olanzapine or clozapine. The present results in rats are in good agreement with a recent study showing that olanzapine can rapidly cause insulin resistance and impaired β-cell compensation in dogs without weight gain.[4] Taken together these data support the hypothesis that the unique pharmacological properties of clozapine and olanzapine produce a direct effect on glucose homeostasis, independent of adiposity.[2] [1] *Consensus Development Conference, Diabetes Care 27: 596-601.* [2] *Newcomer (2005) CNS Drugs 19,Suppl. 1: 1-93.* [3] *Johnson et al (2005) Diabetes 54: 1552-1558.* [4] *Ader et al (2005) Diabetes 54: 862-871.*

109. Alteration of Amygdala Inputs to Entorhinal Cortex Through Dopaminergic Modulation of Voltage-Gated Ion Channels

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: The amygdala in known to have a significant part in the regulation of emotion and memory. One of the primary outputs of the amygdala goes to the entorhinal cortex (EC), a region involved with consolidation and recall of memories. Both of these regions have been reported to display functional or anatomical abnormalities in patients with schizophrenia. Furthermore, dopamine (DA) in these regions can potently modulate formation and recall of memories. However, the physiological actions of DA in the EC have not been examined in depth.
Methods: In this study we utilized in vitro whole cell recordings from the soma and dendrites of neurons of the rat EC to examine how DA modulates their excitability, and inputs from the amygdala.
Results: We found that DA enhanced synaptic integration while reducing membrane excitability. This effect displayed a voltage dependence such that the enhancement of synaptic integration was more prominent at depolarized membrane potentials, whereas the reduction of membrane excitability was more prominent when action potentials were evoked from potentials closer to resting values. Our results indicate that this effect is likely due to DA-mediated enhancement of sodium channel activity, which is apparent only at depolarized potentials, and an enhancement of h-channels, which is not obvious at depolarized potentials. This results in an effect of DA that will depend upon the state of the neuron, whereby large coordinated inputs, or inputs that arrive during a depolarized state will be enhanced by DA. Furthermore, our data demonstrate that DA modulates dendritic information processing.
Discussion: Modulation of voltage-gated ion channels in the dendrites of EC neurons can potently alter synaptic integration and plasticity. This may be one means by which DA modulates memory, particularly those with emotional content that are likely to be influenced by inputs from the amygdala. Abnormal regulation of cortical DA, as may occur in schizophrenia, will be expected to disrupt this aspect of emotional behavior.

110. Antipsychotic-Like Effects of SCA-136: A Novel 5-HT_{2C} Receptor Agonist

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Sponsor: Nancy Ator

Background: Neurochemical studies have shown that activation of 5-HT_{2C} receptors preferentially decreases levels of mesolimbic dopamine (DA) relative to nigrostriatal DA. Given the role of mesolimbic DA in mediating the efficacy of antipsychotics and nigrostriatal DA in mediating the extrapyramidal side-effects of antipsychotics, the present studies investigated if SCA-136 could produce antipsychotic-like efficacy without having the liability of producing extrapyramidal motor symptom side effects.
Methods: SCA-136 was evaluated in animal models predictive of either antipsychotic efficacy (conditioned avoidance responding, apomorphine-induced climbing, nucleus accumbens DA, number of spontaneously active A10 DA neurons/track) or extrapyramidal side-effect (EPS) liabilities (apomorphine-induced stereotypy, catalepsy, striatal DA, number of spontaneous A9 DA neurons/track). In the conditioned avoidance model, SCA-136 was evaluated in a shuttle box avoidance procedure and effect on the number of trials in which there was avoidance, escape, or no response was recorded. In the apomorphine-induced climbing/stereotypy model, following administration of 1 mg/kg apomorphine, the ability of SCA-136 to block either apomorphine-induced climbing or apomorphine-induced stereotypy was recorded. In microdialysis studies, microdialysis probes were implanted either in the nucleus accumbens or striatum, and effects on DA levels in both brain regions were measured following administration of SCA-136. In electrophysiology studies, microelectrodes were placed in either the ventral tegmental area (A10) or the substantia nigra (A9) and the number of spontaneously active DA neurons/track were counted.

Results: SCA-136 produced dose-dependent decreases in conditioned avoidance responding in rats at doses that did not affect the number of no-response trials, a profile predictive of antipsychotic efficacy (MED = 1.7 mg/kg IP). SCA-136 produced dose-dependent decreases in apomorphine-induced climbing with no effect on apomorphine-induced stereotypy (MED = 5.4 mg/kg IP) and did not induce catalepsy in mice, indicative of atypical antipsychotic-like effects with low EPS liability. SCA-136 (17 mg/kg SC) preferentially decreased nucleus accumbens DA (-39%) relative to striatal DA (no change), indicative of mesolimbic selectivity. Acute and chronic administration of SCA-136 (3-17.8 mg/kg IP) decreased the number of spontaneously active A10 DA neurons without affecting the number of spontaneously active A9 DA neurons, predictive of mesolimbic selectivity, rapid onset, and an atypical antipsychotic-like profile.
Discussion: SCA-136 is a novel and potent 5-HT_{2C} receptor agonist (Ki = 3 nM; Rosenzweig-Lipson et al., this meeting). The present studies demonstrate that SCA-136 produces antipsychotic-like efficacy without EPS liability, indicative of an atypical antipsychotic. The acute effects of SCA-136 on both nucleus accumbens DA and on spontaneously active A10 dopamine neurons are suggestive of rapid onset antipsychotic-like effects.

111. Efficacy of Ziprasidone in Schizophrenic Subjects with Differing Levels of Agitation

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Background: Agitation occurs frequently in patients with schizophrenia and is an important treatment target. The objective of this post-hoc analysis was to evaluate whether the presence of agitation is a potentially important factor mediating response to antipsychotic treatment.
Methods: We analyzed data pooled from 2 randomized, double-blind, fixed-dose, placebo-controlled, 6-week trials of ziprasidone (20mg to 100mg BID) in schizophrenia (N=540), to evaluate treatment response in two subgroups defined by moderately high (PANSS excitement (EC) score > 15, N=182) and low (<15, N=358) levels of agitation symptoms at baseline. Improvement in overall severity of illness was assessed using the CGI-S scale. The main effect of treatment and the interaction between treatment and agitation were evaluated using analysis of covariance.
Results: Improvement in CGI-S (and other standard measures of psychopathology) was seen in both individual and pooled studies. Ziprasidone showed significant improvement in CGI-S score (LOCF) compared with placebo, in subjects with both high (PANSS EC score >15) and low (PANSS EC score < 15) levels of agitation symptoms. Mean baseline to endpoint change in CGI-S score was -0.53 for ziprasidone and +0.09 for placebo compared to -0.55 for ziprasidone and -0.31 for placebo for the high and low agitation level subgroups, respectively (P=0.035). Within the ziprasidone arm, improvement in the overall severity score was similar in both subgroups. The placebo-corrected effect size for CGI-S score however, was 0.63 and 0.24 for high and low agitation subgroups respectively. A significant quantitative-interaction effect on CGI-S severity score was observed between treatment (ziprasidone versus placebo) and the level of agitation symptoms at baseline (P=0.039).
Discussion: The effectiveness of ziprasidone for improving overall severity of illness was seen in patients with both low and high levels of agitation, thus indicating overall robust efficacy. Further, the results of an interaction analysis suggests significantly greater effect size for ziprasidone in patients with higher level of agitation symptoms and that placebo response in patients with high PANSS EC scores is low, Agitation may be an important variable mediating treatment response to ziprasidone. Whether this is true for all antipsychotic medications, for atypicals in general or is specific to zipasidone will require further study.

112. A Pharmacogenetic Test for Prediction of Vulnerability to Tardive Dyskinesia

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Sponsor: Arieh Shalev

Background: Tardive dyskinesia (TD) affects up to 49% of schizophrenia patients following chronic exposure to dopamine receptor antagonist drugs. Drug and patient related risk factors account for a small portion of the variance in the incidence of TD. In addition, a number of polymorphic genes have been associated with small increments in risk for TD. While supporting a polygenic multifactorial model modulating TD vulnerability, the magnitude of each of these effects precludes their use to guide clinical practice.
Methods: We applied a predictive model based on the cumulative contribution of genetic, clinical, and demographic variables to dyskinesia scores as rated by the Abnormal Involuntary Movements Scale (AIMS), employing a case control association design among 121 chronically medicated schizophrenia patients.
Results: Multiple regression analysis found a number of factors that showed signficant and independent impact on AIMS scores. These included genotypic data from five polymorphic genetic loci including the dopamine D3 receptor (DRD3), serotonin receptors HTR2C and HTR2A, magnesium superoxide dismutase (MNSOD) and the metabolic enzyme cytochrome p 17 alpha hydroxylase (CYP17). Demographic and clinical variables that impacted signficantly on risk were the patient's age at first antipsychotic drug treatment, total score on the positive and negative symptom scale (PANSS), drug dosage in chlorpromazine equivalent units, age, and gender. A step wise regression model based on these factors correctly classified 72% of patients with clinical TD (LR Chi2 (df 9) = 28.53, p = .0008; sensitivity 72.3% specificity 71.7 %, false positive rate 28.2%, false negative rate 27.6%).
Discussion: The level of sensitivity and specificity achieved provides proof of concept for the utility of a multifactorial pharmacogenetic test for prediction of susceptibility to TD. Replication in indepent samples is required to generalise the predictive utility of these factors. Further development of the test should include additional genetic polymorphisms and clinical variables and thereby increase its predictive power and clinical utility.

113. Neuregulin1-Induced Cell Migration of B Lymphoblasts: Possible Association with Schizophrenia

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Sponsor: Daniel Weinberger

Background: Neuregulin1 (NRG1) plays a critical role in neuronal migration and thus the development of the central nervous system. It has been suggested that signaling mediated by NRG1 is associated with neuropathogenesis of Schizophrenia. Since neuronal cells and immune cells share many cellular and molecular mechanisms for cell migration, we tested NRG1-induced cell migration using B lymphoblasts from patients.
Methods: Expressions of ErbB2, 3 and 4 were examined by RT-PCR, Western blot and immunohisotochemistry. Phosphorylation status of AKT and ERK was monitored by Western. Intracellular calcium levels was measured by flow cytometry using fluo-3. Cell migration was assayed using 96-Transwell assay kits from Chemicon and Calbiochem.
Results: We found that NRG1 induced cell polarization, co-localization of ErbB2 and ErbB3 receptors and actual cell migration. ErbB2-specific inhibitor AG825 abrogated NRG1 effects on migration, indicating that NRG1-induced migration was mediated by ErbB2

receptor. Further, we observed that NRG1 increased phosphorylation of ERK and AKT and intracellular calcium levels, suggesting that NRG1 activated MAPK, PI3K and PLC-gamma signaling pathways in B lymphoblasts as commonly observed in neuronal cells. Studies using kinase inhibitors suggested that NRG1-induced migration of B lymphoblasts required activations of PI3K and PLC-gamma but not MAPK. Finally we found that NRG1-induced migration of B lymphoblasts in patients with Schizophrenia (n=13) was significantly decreased than those in control individuals (n=13) (p=0.0071, Mann-Whitney test). This observation was further validated in a new group of 32 subjects. Thus, we confirmed again that the cell migration to NRG1 was significantly lower in patients with Schizophrenia (n=12) than those in controls (n=20) (p=0.0006, Mann-Whitney test).
Discussion: Overall results suggest that cell migration mediated by NRG1-ErbB signaling is impaired in patients with schizophrenia. Our findings may provide a novel insight on neurodedelopmental hypothesis of schizophrenia.

114. Differential Methamphetamine-Induced Immediate Early Gene Expression in the Prefrontal Cortex of High Versus Low Responders

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Sponsor: John Kelsoe

Background: The molecular mechanisms underlying individual differences in sensitivity to methamphetamine (METH) administration are not well understood. METH administration can produce many of the symptoms of mania and often induces psychosis after chronic treatment. Therefore, the genetic mechanisms that contribute to differences in sensitivity to METH may also facilitate the development of mania and psychosis.
Methods: To investigate the potential mechanisms that contribute to individual susceptibility, we treated 50 Sprague Dawley rats acutely with METH (4.0 mg/kg) and 10 control rats with saline. Behavior was measured for three hours after drug administration. Animals were divided into high responders (HR) (top 20%) and low responders (LR) (lowest 20%) based on the large individual differences in their stereotypy response to METH treatment. In this regard, HR exhibited a decreased latency to stereotypy and a longer duration of focused stereotypy compared to the LR. Rats were sacrificed twenty-four hours after METH injection. Total RNA was extracted from the prefrontal cortex (PFC) and Affymetrix 230 2.0 GeneChips were used to analyze the expression of approximately 30,000 genes. Raw images were analyzed and features extracted using GCOS 1.1 (Affymetrix, Foster City, CA). The resulting CEL files were then normalized and converted to gene intensity values by Robust Analysis of Microarrays (RMA). Data were analyzed by ANOVA and Student-Neuman-Keuls post hoc tests (Genespring, Silicon Genetics). The expression of a select group of genes was validated by real time reverse transcription polymerase chain reaction (RT-PCR).
Results: Immediate early genes including c-fos (- 45%), ARC (- 35%), NGFI-B (- 22%), and jun-B (- 18%) were significantly down-regulated in the HR but not in the LR suggesting a differential METH-induced responsiveness of signal transduction pathways in these two groups of rats. These immediate early genes also exhibited significant expression differences in HR compared to LR. In addition, a subset of the genes that were up-regulated in LR and but not in HR, e.g., serum/glucocorticoid regulated kinase (39%), and CAAT/enhancer binding protein (23%) also exhibited significant differences in expression in HR compared to LR.
Discussion: These differences in METH-induced gene expression in the PFC of HR versus LR may contribute to individual differences in drug sensitivity and the development of mania and psychosis. 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."

115. Disruption of Startle Gating in Rats After Systemic D1 Blockade Is Independent of Prefrontal Cortex and Changes in Sensory Gating

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Sponsor: Neal Swerdlow

Background: Hypofunction in frontal D1 systems has been implicated in the pathophysiology of schizophrenia. This abnormality might contribute to sensorimotor and sensory gating deficits in schizophrenia, based on evidence that reduced D1 transmission in the medial prefrontal cortex (MPFC) disrupts prepulse inhibition of startle (PPI) in rats. We studied the neural basis for reduced PPI in rats after systemic administration of the D1 antagonist, SCH23390. In separate animals, we also examined whether this loss of gating could be detected via measures of cortical auditory-evoked potential (AEP) N40 gating.
Methods: Acoustic startle and PPI were measured in rats after intra-MPFC and systemic administration of SCH23390. In other rats, the effects of systemic administration of SCH23390 on PPI were tested 1-2 weeks after either sham or ibotenic acid lesions of the MPFC. Lastly, the gating-disruptive effects of D1 blockade were assessed in rats during contemporaneous measures of PPI and AEP gating, using a paired-click paradigm to assess N40 AEP suppression. Data were analyzed by mixed design ANOVAs, with alpha = 0.05.
Results: PPI was disrupted by both systemic administration of SCH23390 (0.1 mg/kg sc), and by direct intra-MPFC administration of SCH23390 (3_{μ}g/side); in both cases, these effects on PPI could be clearly distinguished from drug-induced changes in startle magnitude on pulse alone trials. The PPI-disruptive effects of systemic SCH23390 were not opposed by ibotenic acid lesions of the MPFC. ANOVA revealed a significant effect of SCH23390 dose (0 vs. 0.1 mg/kg sc; $p<0.0001$), but no significant effect of lesion or dose x lesion interaction. Contemporaneous measurement revealed that D1 blockade disrupted PPI (main effect of dose, $p<0.0005$) but not N40 AEP gating ($F<1$); findings revealed reduced amplitude of both startle and AEP (S1) responses after D1 blockade.
Discussion: In several experiments, we confirmed that D1 receptors regulate PPI in rats, and that D1 blockade in the MPFC disrupts PPI. However, the loss of PPI after systemic administration of SCH23390 does not appear to be mediated by D1 blockade in the MPFC, nor does it appear to be accompanied by a loss of sensory gating, as detected by cortical AEP suppression. Supported by MH53484, MH01436 and MH42228.

116. Frequency Mismatch Negativity in Mice is Attenuated by Ketamine

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Background: Mismatch negativity (MMN) is a deviance elicited auditory event-related potential (ERP). The current report is an exploratory study to find MMN in mice using a balanced frequency deviant task and examine the effects of NMDA antagonism on this measure.
Methods: ERPs were recorded in awake and alert DBA2 mice prior to and following 10 mg/kg ketamine administration. The deviance protocol was created to eliminate proposed confounds that have prevented frequency MMN detection in previous mouse studies.
Results: There was significantly larger area under the curve (AUC) for the MMN component after deviant tone compared to standard

following vehicle. This increase was abolished following ketamine treatment.
Discussion: The balanced frequency task elicited a MMN in DBA mice that was attenuated with exposure to the NMDA antagonist ketamine. We demonstrate that the pattern of subtle and random frequency differences for deviant tones used in our paradigm is well suited for evoking MMN in mice. This demonstration of frequency MMN in mice provides a foundation for future studies examining sensory processing deficits in response to deviant stimuli. These findings have implications for future mouse models of psychiatric disorders including schizophrenia.

117. Metabolic Responses to Fatty Meal in Schizophrenic Patients Treated with Olanzapine or Risperidone
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Background: Second generation antipsychotic drugs have been reported to be associated with increased risk of diabetes related glucose and lipid metabolic abnormalities. Some studies have suggested that olanzapine treatment may be particularly associated with increases in triglycerides or lipids. Metabolic responses to a Fatty Meal (FM) have been used to study postprandial glucose and lipid metabolism in normals and diabetics, and may be more sensitive to exploring metabolic changes induced by drugs or disease states.
Methods: In the context of a 5-month random assignment metabolic study of treatment with olanzapine or risperidone in chronically hospitalized schizophrenic patients, we performed a fasting FM test (1.2 g fat, 1.1g carbohydrate, 0.2 g protein corrected for BMI) in the morning, at a)baseline - before randomized drug assignment - and b)after 2 months of treatment with randomzied study drug- olanzapine or risperidone. We assayed serum glucose, insulin, free fatty acid (FFA), cholesterol, triglyceride, VDL (very low density) cholesterol, and serum ghrelin (a gut hormone related to central appetite regulation) before administration of FM and at 1 and 4 hours after FM.
Results: After 2 months of study drug treatment the FM raised triglycerides, VLDL and insulin at 1 and 4 hrs post FM, and glucose at 1 hr post FM, but did not significantly change cholesterol, FFA or HDL and slightly decreased LDL. A similair pattern was seen after baseline FM test. At the 2 month time point there were no significant differences between effects of olanzapine and risperidone on FM test responses on any metabolic measure in the overall ANOVA's (i.e.- no significant F's for drug x time interaction). Within subject contrasts revealed a trend ($P<.07$) for VLDL increases to be slightly greater for olanzapine than risperidone at the 4 hr time point, and the increase in insulin to be greater ($p<.04$) for olanzapine the 1 hr time point. Ghrelin decreased about 11% 1 hr after FM and returned to pre FM same-day baseline by 4 hours. There were no differences between olanzapine vs. risperidone in ghrelin levels at any time point at pre randomization baseline or after 2 months of study drug treatment. There were no drug differences in changes in ghrelin levels after FM. Ghrelin response did not correlate with baseline BMI or weight change during study drug treatment.
Discussion: Our results suggest fairly similar glucose and lipid responses to FM test in chronic schizophrenic inpatients treated with olanzapine or risperidone, and no significant difference in triglyceride response.

118. Effects of Smoking and Nicotine Nasal Spray on Cognitive Function in Schizophrenic Smokers, Schizophrenics Non-Smokers, and Non-Psychotic Smokers
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Background: Schizophrenics have a very high rate of cigarette smoking, with rates in hospitalized inpatients approached 80-95% in some studies, and it has been hypothesized that nicotinic deficits, including changes in brain nicotinic receptors, may be involved in the pathophysiology of schizophrenia. Our group and others have previously reported that smoking or nicotine administration improves performance of schizophrenics on some neurocognitive tests.
Methods: We are presenting preliminary resutls from a larger study of the effects of smoking cigarettes and/or receiving nicotine nasal spray on neurocognitive tests of attention-vigilance (CPT), visual spatial memory (Dot Test and ANAM figure rearrangement), and verbal memory (RANDT), in a placebo controlled double-blind study of schizophrenic smokers, schizophrenic non-smokers, and non-psychotic control smokers. Subjects participated in 4 to 8 sessions, conducted on different days, in which they were tested both before and after active or placebo cigarette or nicotine nasal spray administration, on neurocognitive tests, and for blood pressure, pulse, and nicotine-cotinine levels.
Results: Chronic schizophrenics who were cigarette smokers showed decreased reaction time (RT) on CPT in active cigarette and nasal spray sessions. They also showed increased percent correct responses and deceased percent omissions in active cigarette sessions, and trends for improvement in visual spatial memory with active nasal spray (ANAM visual rearrangement task - increased accuracy /time, and in Dot task - decreased difference between delayed and immediate recall). Nicotine nasal spray did not have a strong effect on improving verbal memory in schizophrenic smokers. Cigarette smoking in these patients also did not produce improvement in RANDT scores. In schizophrenics who were not cigarette smokers active nicotine nasal spray did not decrease RT and worsened performance on some measures in CPT task; these schizophrenic non-smokers showed a small non- significant trend for improvement performance on the two visual spatial tasks in the active nasal spray session. These non-smoking schizophrenics generally did show slightly improved scores on RANDT total and paired words sub-test afer active nasal spay. Non-psychotic control smokers administered active or placebo nasal spray, showed overall improved performance scores on most cognitive tests compared to schizophrenic patients, but showed no effects of nicotine nasal spray on most measures of CPT performance. There was no effect of nicotine nasal spray on visual spatial memory in Dot task in this group, but improved accuracy in the active nasal spray sessions in the ANAM spatial reorganization task. They did not show significantly improved performance on total RANDT scores with active nasal spray but did have small but significant improvement in the paired words subtest.
Discussion: These preliminary resutls suggest modest cognitive effects of cigarette smoking and/or nicotine nasal spray on some neurocognitive measures related to attention and visual-spatial memory schizophrenics, and some differential effects of nicotine nasal spray on cognitive function in schizophrenic smokers, schizophrenic non-smokers and non-psychotic control smokers.

119. Effectiveness and Tolerability Outcomes of Risperidone Long-Acting Injection Compared to Conventional Depot Antipsychotics in a Canadian Psychiatric Hospital
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Sponsor: John Csernansky

Background: There is a pausity of data directly comparing effectiveness and tolerability of atypical and conventional long-acting antipsychotics. The authors objective was to compare effectiveness and tolerability outcomes of patients with schizophrenia treated with risperidone long-acting injection and patients treated with conventional depot antipsychotics in an observational and naturalistic setting.
Methods: Patients initiated on risperidone long-acting injection during a 4-month index period were compared to patients initiated on a conventional depot antipsychotic during the same time period. Pa-

tient demographics including age, gender, diagnosis, number of previous psychiatric admissions and inpatient program were compared. The use of antipsychotic polypharmacy as well as discharge and readmission rates were evaluated. Tolerability was assessed by measuring the prescribing rates of regularly scheduled anticholinergic rescue medications.
Results: 40 patients initiated on risperidone long-acting injection were compared to 49 patients initiated on a conventional depot antipsychotic. The two patient groups were demographically very similar. The risperidone long-acting injection group was 75% male with an average age of 41-years and 6.0 previous psychiatric admissions. The conventional depot group was 67% male with an average age of 47.5 years and 5.9 previous admissions. Antipsychotic polypharmacy was reduced from 63% to 31% in the risperidone long-acting injection group but increased from 29% to 73% in the conventional depot group. The use of anticholinergic rescue medications decreased from 47% to 12% in the risperidone long-acting injection group but increased from 31% to 73% in the conventional depot group. After 12-months of observation, 83% of the risperidone long-acting injection group had been discharged from hospital and none had been readmitted, whereas 58% of the conventional depot group had been discharged and, of those, 26% had already been readmitted.
Discussion: In this difficult-to-treat population of patients, risperidone long-acting injection conferred significant advantages over conventional depot antipsychotics in terms of effectiveness and tolerability. As well, the substantial differences in discharge and readmission rates suggest considerable pharmacoeconomic advantages in favor of risperidone long-acting injection.

120. Evidence for Epigenetic Regulation of the 5HT2A Receptor Gene (5HT2AR). Methylation of Allele C-Specific CpG Sites in 5HT2AR Correlates with its Expression and eRobust, Rapid and Relatively Sustained Antidepressant Effects with a Single-Dose of an NMDA Antagonist in Treatment-Resistant Major Depression: A Double-Blind Placebo-Controlled Study Expression of DNA Methylase DNMT1

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Sponsor: Robert Schwarcz

Background: Hypoactivity of allele C of the serotonin 5HT2A receptor gene (5HT2AR) and genetic association between this allele and several psychiatric disorders have been reported but not universally confirmed. Differential DNA methylation has been suggested to contribute to differential activity of alleles C and T and thereby to the complexity of genetic associations between allele C and psychiatric disorders. Here we surveyed methylation in two allele C-specific CpG sites and examined its relationship with the expression of 5HT2AR and expression of DNA methylase DNMT1.
Methods: Highly specific assays based on methylation-sensitive restriction-polymerase chain reaction (MSR-PCR) were developed to detect and measure methylation of allele C-specific CpG sites in the 1st exon (at 102/3) and in the promoter (at -1437/8) of the 5HT2AR gene. DNA methylation was measured in the temporal cortex (BA21) from 10 normal individuals and leukocytes from 18 normal individuals. Levels of 5HT2AR and DNMT1 mRNA were measured using microarrays and RT-PCR.
Results: The majority of allele C-specific CpG sites were methylated in the temporal cortex and peripheral leukocytes. Levels of methylation of allele C-specific CpGs in the 1st exon varied in the brain significantly between individuals (40% to 87%, ANOVA, $p=0.003$). Methylation levels in the 1st exon and in the promoter were significantly correlated ($r=0.77$, $p=0.009$). Levels of methylation in the promoter correlated significantly ($r=0.680$, $p=0.031$) with the expression of 5HT2AR. Methylation of allele C-specific CpG sites in the 1st exon correlated significantly ($r=0.649$, $p=0.021$) with the expression of DNA methylase 1 (DNMT1) but not S-adenosylhomocysteine hydrolase (AHCY).
Discussion: These findings support the hypothesis that allele-specific DNA methylation is involved in regulation of 5HT2AR influencing expression differences between alleles C and T. We hypothesize that allele C-specific CpG site in the promoter of 5HT2AR may create a repressor site that causes allele C hypoactivity. DNA methylation may neutralize this repressor site reducing differences in expression between alleles C and T. Individual variations in DNA methylation may therefore cause variations in allele C hypoactivity, explaining why allele C hypoactivity and its association with psychiatric disorders were found in some populations but not in others. The findings here demonstrate that a genetic polymorphism may result in epigenetic differences between alleles suggesting complex interactions between genetic, epigenetic and environmental factors in regulation of the 5HT2AR gene.

121. Deficit Syndrome and Memory Function in Schizophrenia

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Sponsor: Travel Awardee, Pfizer, 2005

Background: Schizophrenia is a syndrome characterized by domains of dysfunction, including psychosis, cognitive defects and negative symptoms. The biology implicated for each of these domains includes the prefrontal cortex (PFC) and temporal lobe for psychosis and cognition and PFC and parietal cortex (PC) for negative symptoms. Declarative memory in particular is supported by cerebral systems that include the PFC, medial temporal lobe (MTL) structures and basal ganglia (BG), partially overlapping with areas thought to be involved in negative symptoms. The deficit syndrome is characterized by primary enduring negative symptoms that predict poor outcome and low socio-occupational functioning. Since cognitive impairment may contribute to functional deficits, we sought to examine the influence of deficit symptomatology on memory systems in schizophrenia.
Methods: We have compared deficit with non-deficit schizophrenia subtypes using three MTL-dependent tasks: novelty detection, transitive inference, and acquired equivalence. We have evaluated performance after these three tasks and cerebral activation patterns using fMRI BOLD with novelty detection and subsequent memory. Earlier studies have suggested that deficit patients differ from non-deficit patients on frontal and parietal cognitive tasks (Buchanan et al 1994; Brazo et al 2002; Horan and Blanchard 2003) and have resting hypometabolism in prefrontal and parietal regions (Tamminga et al 1992). Therefore, it was our a priori hypothesis that the deficit schizophrenia group would show greater decrements in performance than the non-deficit group and would show reduced BOLD activation in PFC (in both primary and compensatory activations) during novelty detection.
Results: We have already demonstrated alterations in conjunctive memory performance in schizophrenia, across cognitive dimensions and diverse symptoms. Moreover, in novelty detection, we have also shown reduced left hippocampal activation during novel stimuli (scenes) in schizophrenia.
Discussion: We will report the impact of deficit vs non-deficit state on novelty detection and memory performance in schizophrenia. These data will contribute to the characterization of memory function in schizophrenia subgroups.

122. MUTED (6p24.3), a Protein That Binds to Dysbindin (DTNBP1, 6p22.3), Is Strongly Associated with Schizophrenia and Exhibits Statistical Epistasis with COMT

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Background: Using known schizophrenia susceptibility genes as leads into functional pathways could prove an efficient way of identi-

fying novel etiological genes and pathways. Dysbindin is probably a component of two macromolecular complexes in brain: 1) the BLOC-1 (biogenesis of lysosome-related organelles) complex and 2) the DPC (dystrophin protein complex). We tested SNPs in genes coding for other proteins in these complexes.
Methods: Family based tests (TDTPHASE, FBAT) of association with the clinical phenotype of schizophrenia were performed in the Caucasian families in the NIMH Sibling Study. We tested 52 SNPs in dysbindin, and multiple SNPs in BLOC-1 complex members MUTED, PLDN, SNAPAP, GCN5L1, BLOC1S3, and CNO and in genes that appear from proteomic and functional studies to biologically interact with BLOC-1, including EEA1, STX6, STX12, and SNAP25. We also tested at lower SNP density some DPC components (DTNB, SGCD, SGCE) and related proteins (DAG-1, ACTN2). We tested for interaction between COMT val158met genotype and genotypes at SNPs in MUTED by logistic regression, using the affected status of cases and controls as the outcome variable.
Results: MUTED was strongly associated with the clinical phenotype: 5 of the 17 SNPs tested were positive (p=0.028-0.0002), as were their haplotypes. Of 12 EEA1 SNPs, 3 were positive (p=0.037-0.050). No other gene yielded more than one positive SNP. In addition, conditioning the analysis of dysbindin on either of two of the MUTED SNPs gave far more significant dysbindin results than without conditioning, which is likely to reflect some combination of locus heterogeneity and epistasis. Finally, logistic regression yielded evidence of statistical epistasis between COMT val158met and multiple SNPs in MUTED (p=0.011-0.050).
Discussion: This preliminary survey of genes functionally related to dysbindin suggests that BLOC-1 dysfunction may be more important than DPC dysfunction in increasing the risk of developing clinical symptoms. However, this interpretation is quite tentative, and certainly does not rule out a role for the DPC in the cognitive deficits associated with schizophrenia and with variation in dysbindin. More detailed genetic analysis of both complexes, coupled with more comprehensive analyses for epistatic interactions between other combinations of genes, is in progress. Dysbindin is downregulated presynaptically in glutamatergic hippocampal neurons of schizophrenics (Ref 1), and this reduction appears to inhibit glutamate release (Ref 2). The evidence from these studies suggests that this inhibition is mediated via reduced BLOC-1 complex functioning. Our statistical epistasis results with COMT and the dysbindin binding partner MUTED may be due to some aspect of the crosstalk between dopamine and glutamate signaling that is relevant to schizophrenia susceptibility. 1) Talbot K. et al , J.Clin Invest 13:1353-1363 (2004); 2) Numakawa T. et al, Human Molec Genet 13:2699-2708 (2004).

123. Bifeprunox: Relationship Between Antipsychotic Potential, EPS Liability and Dopamine D2 Receptor Occupancy in Rats

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Background: Positron emission tomography has shown that antipsychotic response and extrapyramidal side effects (EPS) of D2 receptor antagonist antipsychotics are related to striatal dopamine (DA) D2 receptor occupancy, albeit with different thresholds. Overall, the antipsychotic effect of D2 receptor antagonists occurs within the range of 40-80% D2 receptor occupancy while EPS incidence increases above the 80% threshold. These values closely resemble results from preclinical assessment of antipsychotic efficacy and EPS liability using the conditioned avoidance response (CAR) model and measurement of cataleptic potential, respectively. Thus, D2 receptor blocking antipsychotics suppress CAR within a range of 40-80% D2 receptor occupancy whereas cataleptic behaviour occurs at D2 receptor occupancies above 80%. It is not clear whether such a relationship also applies for partial D2 receptor agonists.
Methods: Bifeprunox (7-[4-([1,1'-biphenyl]-3-ylmethyl)-1-piperazinyl]-2(3H)-benzoxazolone, monomethane-sulphonate) is a novel putative antipsychotic agent which possesses partial agonist effects at dopamine D2 and serotonin 5-HT1A receptors. This study assessed the relationship for bifeprunox between in vivo D2 receptor occupancy and its antipsychotic activity measured by suppression of CAR and, the ability to antagonize amphetamine (AMPH)-induced hyperactivity. The relation between D2 occupancy and EPS potential was investigated by measuring induction of catalepsy. CAR was assessed in automated, two-compartment shuttle boxes while animal cages equipped with infrared light sources and photocells were used for AMPH-induced (0.5 mg/kg, s.c.) hyperactivity measurements. Cataleptic behaviour was scored manually by placing animals on vertical wire netting. In vivo D2 receptor occupancy was measured using [3H]raclopride as radioligand and an immunohistochemical procedure with polyclonal anti-c-Fos antibodies employed to assess induction of striatal c-Fos expression. Bifeprunox effect in all assays was measured 2 hr after s.c. administration.
Results: Bifeprunox suppressed CAR without inducing escape failures in rats with a minimum effective dose of 0.25 mg/kg, s.c. In this dose-range, the D2 receptor occupancy was 100%. Bifeprunox antagonized AMPH-induced hyperactivity at doses much lower than those suppressing CAR (ED50=0.0050 mg/kg, s.c.) with corresponding receptor occupancy of 30%. Bifeprunox did not induce catalepsy signs in rats within the tested dose range (up to 16 mg/kg, s.c.). Low EPS potential was further underlined by finding that bifeprunox did not induce striatal c-Fos expression at dose levels significantly suppressing CAR (up to 1.56 mg/kg, s.c.).
Discussion: This data shows bifeprunox has antipsychotic-like effects. In contrast to conventional D2 receptor antagonist antipsychotics, bifeprunox effects in the CAR model occur at maximal, or near maximal, D2 receptor occupancy. Importantly, despite the high degree of D2 receptor occupancy, no markers for EPS potential were observed for bifeprunox, which contrasts with conventional D2 receptor antagonist antipsychotics. In addition, bifeprunox more potently inhibited behaviour induced by hyperdopaminergia (AMPH-induced hyperactivity). A likely explanation for these differences is that the partial agonist effect of bifeprunox at high D2 receptor occupancy is associated with antipsychotic activity, without EPS liability.

124. Enhanced Cortical Dopamine Output and Antipsychotic-Like Effect of Raclopride by Low Dose L-Dopa

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Background: Clozapine shows superior efficacy in schizophrenia and enhances like other atypical, but not typical, antipsychotic drugs (APDs) prefrontal dopamine (DA) output. Clinical data also demonstrate an improved effect of typical APDs in schizophrenia by adjunctive treatment with low doses of L-dopa, but experimental support is scarce and the underlying mechanisms are poorly understood.
Methods: Antipsychotic efficacy of the D2 antagonist raclopride with or without adjunctive treatment with a low dose of L-dopa was assessed using the conditioned avoidance response. Extrapyramidal side effects were scored by the catalepsy test. Finally, in vivo microdialysis was used to measure DA efflux in the prefrontal cortex and the nucleus accumbens, respectively.
Results: : A low dose of L-dopa (3 mg/kg) combined with benserazide, an inhibitor of peripheral DOPA decarboxylase, significantly enhanced the antipsychotic-like effect of raclopride without any associated catalepsy. L-dopa/benserazide alone had no effect. In contrast to raclopride alone, combined L-dopa/raclopride also produced a much larger increase in prefrontal DA output than in the nucleus accumbens.
Discussion: These data support the clinical observation that low dose L-dopa improves the effect of typical APDs in schizophrenia and propose that an increased prefrontal DA output per se enhances the clinical effect of typical APDs, and in principle generate a clozap-

ine-like clinical profile, with enhanced effect on negative and cognitive symptoms.

125. Effects of Memantine on Startle Gating in Normal Adult Men

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Background: Reduced sensorimotor gating (prepulse inhibition: PPI) exhibited by schizophrenia patients is reproduced in rats treated with NMDA receptor antagonists; this supports the hypothesis that reduced glutamate function may contribute to the pathophysiology of schizophrenia. However, compared to PPI in rats, the regulation of PPI by NMDA in humans is less well understood. Studies with amantadine and ketamine have yielded mixed results, but the weight of the evidence suggests that NMDA blockade in normal humans actually increases, rather than decreases, PPI. Neither amantadine nor ketamine are optimal for studying NMDA function in humans, based on amantadine's low NMDA affinity and mixed DA agonist properties, and on ketamine's psychotomimetic properties. Here, we tested the effects of the low-to-moderate affinity NMDA antagonist, memantine, on PPI in normal men.
Methods: This study was approved by the UCSD Human Subjects IRB and conducted in accordance with NIH guidelines. 20 carefully screened normal right handed 18-35 yo men were free from psychiatric, medical or substance disorders, and had robust startle reflex magnitude and PPI. Subjects were tested twice, after placebo or 20 mg memantine po, in a double-blind crossover design. Testing included electromyographic (EMG) measures of acoustic startle and PPI, autonomic monitoring, and self-rating scales. Startle screening and testing included 42 trials with 6 conditions: a 118 dB(A) 40 ms noise burst presented alone (P-ALONE); and the same 118 dB(A) 40 ms noise burst preceded 10, 20, 30, 60 or 120 ms by a prepulse (5 ms noise burst) 16 dB above background. Testing began 210 min after placebo or memantine administration, based on published time course data for peak memantine blood levels, and was repeated later in the day; data reported here are from the initial test session. Data from one subject could not be analyzed due to low startle magnitude.
Results: Memantine (20 mg) caused no significant change in either heart rate or blood pressure, or subjective ratings of perception, drowsiness or nausea. Compared to placebo, subjects rated their mood as more "happy" during min 200 - 230 after memantine, based on ratings taken pre- and post-PPI testing. P-ALONE startle magnitude was unaffected by memantine. ANOVA of PPI revealed a significant interaction of prepulse interval x dose, reflecting a significant memantine-induced increase in PPI for 120 ms prepulse intervals ($p<0.025$). Post-hoc comparisons revealed that this effect was dependent on baseline (pre-drug) PPI levels: subjects with low baseline PPI exhibited memantine-induced increases in 120 ms PPI, while those with high baseline PPI did not, and actually exhibited drug-induced reductions in short-interval PPI.
Discussion: Similar to previous reports with amantadine and ketamine, 20 mg memantine increased PPI in normal adult men. This effect was limited to a time point associated with maximal blood levels and bioactivity based on mood ratings; it was observed only at 120 ms prepulse intervals and was most evident in subjects with low basal PPI levels. Memantine had mild, time-limited mood enhancing effects but no other effects on autonomic, startle magnitude or subjective measures. These findings support evidence that NMDA blockade in normal humans increases PPI, rather than decreases PPI. Similar to other findings with dopamine agonists and amantadine, this effect appears to be "rate-dependent" (i.e. dependent on basal PPI levels). Studies with a higher dose of memantine (30 mg) are in progress. The present findings make it difficult to explain reduced PPI in schizophrenia patients simply on the basis of reductions in NMDA neurotransmission. Supported by MH59803, MH01436 and 5M01RR000827.

126. Lower Anterior Cingulate Grey Matter Volume in Antipsychotic Drug-Naive First-Episode Schizophrenia Patients with Cannabis Use

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Sponsor: David Rosenberg

Background: Although cannabis use is probably not causally related to schizophrenia expect in a small percentage of individuals, its use might precipitate psychosis among vulnerable individuals and exacerbate the disorder among individuals who are active users. Animal studies suggest that using $\triangle$ 9 Tetrahydrocannabinol (THC), the main psychoactive component of cannabis, may be neurotoxic to the brain. Although numerous studies have identified brain structural abnormalities in schizophrenia, little research has been directed at understanding the potential association between these abnormalities and cannabis use in this disorder. We tested the hypothesis that patients with cannabis use would have greater prefrontal structural abnormalities compared to patients without cannabis use and healthy volunteers
Methods: Fifty-two patients met DSM-IV criteria for schizophrenia (N=37), schizoaffective (N=8) or schizophreniform (N=7) disorder. Of the 52 patients included in this study 4 had a diagnosis of cannabis abuse and 13 had a diagnosis of cannabis dependence. Of the 17 patients with a diagnosis of cannabis abuse or dependence 5 also had a diagnosis of alchohol abuse or dependence. Thirty-one patients were antipsychotic drug-naive at the time of the scan including 8 patients from the group with cannabis use and 22 patients from the group without cannabis use. In addition, 59 healthy volunteers were included in the study as determined by clinical interview and the SCID-NP. MR imaging exams were acquired in the coronal plane using a 3D Fast SPGR with IR Prep on a 1.5 Tesla whole body superconducting imaging system (General Electric, Milwaukee, WI). We measured grey and white matter volumes of the superior frontal gyrus, anterior cingulate gyrus and orbital frontal lobe.
Results: Mixed models analyses revealed a significant group-by-tissue type interaction for the anterior cingulate ($p < .05$) such that patients with cannabis use had significantly less anterior cingulate grey matter compared to patients without cannabis use and healthy volunteers. The group-by-tissue type interaction remained statistically significant ($p < .05$) for the anterior cingulate when we excluded patients from analysis who had any substance use diagnosis other than cannabis abuse or dependence. Further restricting the analysis to antipsychotic drug-naive patients also revealed a significant group-by-tissue type interaction ($p < .05$) yielding a similar set of results.
Discussion: Using methods for cortical parcellation of the prefrontal cortex based on the sulcal anatomy we report that patients experiencing a first-episode of schizophrenia who use cannabis have less anterior cingulate grey matter compared to first-episode patients who do not use cannabis and healthy volunteers. The anterior cingulate is believed to play an important role in mediating executive functions, including set-shifting, response inhibition and decision making, which have been reported to be abnormal in cannabis users. Our results are therefore compatible with the hypothesis that structural alterations involving the anterior cingulate in patients using cannabis could be associated with poor decision making and partly mediate the compulsive drive toward drug use.

127. Distinct Roles for Different Homer1 Isoforms in Behaviors Associated with Prefrontal Cortex Function in vivo

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Sponsor: Past Travel Awardee, Young Investigator Memorial, 2004

Background: Mutational analyses conducted in a large heterogenous population of patients with schizophrenia have identified a mutation in the gene coding the Homer1 members of the Homer family of postsynaptic scaffolding proteins. Consistent with this clinical observation, extensive phenotyping of Homer1 null mutant mice has provided face, predictive and construct validation of this mouse as a genetic animal model of schizophrenia. As the Homer1 gene encodes both immmediate early gene (IEG) and constitutively expressed (coiled-coil; CC) isoforms, it remains to be determined whether different Homer1 isoforms play distinct roles in the regulation of corticofugal glutamate transmission implicated in cognitive, sensorimotor and emotional processing.
Methods: To determine the relative role for IEG and CC-Homer1 gene products in the "schizophrenic-like" behavioral phenotype of Homer1 KO mice, the phenotype of KO mice was "rescued" by prefrontal cortex (PFC) infusion of adeno-associated viral vectors (AAVs) carrying either GFP control, the IEG isoform Homer1a or the CC isoform Homer1c. Subsequent to AAV infusion, Homer1 (wild-type) WT and knock-out (KO) mice were assessed for genotypic differences in cognitive function (radial arm maze), emotionality (reactivity to novelty and Porsolt swim test) and sensorimotor processing (pre-pulse inhibition of acoustic startle). To assess for effects of AAV infusion upon genotypic differences in sensitivity to the behavioral effects of stimulant drugs, a dose-response study for cocaine-induced locomotion was also conducted. Finally, to relate the behavioral effects of Homer1 isoform over-expression to the regulation of corticofugal glutamate, no net-flux in vivo microdialysis assessed for differences in basal glutamate content and conventional in vivo microdialysis assessed for differences in cocaine-stimulated glutamate release.
Results: Intra-PFC infusion of AAV-Homer1a reversed the genotypic differences in behavioral adaptation to repeated stress, whereas infusion of AAV-Homer1c reversed the genotypic differences in sensorimotor and cognitive processing, as well as cocaine behavioral sensitivity. AAV-Homer1a infusion did not influence PFC basal glutamate content, but blunted the glutamate response to cocaine in WT mice. In contrast, Homer1c infusion reversed the genotypic difference in PFC basal glutamate content and enhanced cocaine-induced elevations in glutamate.
Discussion: These data provide the first in vivo demonstration of active and distinct roles for IEG and CC-Homer1 isoforms in the PFC in the mediation of behavior, as well as the maintenance of basal extracellular glutamate in the PFC and the regulation of PFC glutamate release.

128. Asenapine Displays Unique Long-Term Molecular Effects on Dopamine Receptor Subtypes

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Background: Asenapine is a novel psychotherapeutic agent reported to significantly improve treatment of schizophrenia based on an initial study demonstrating improved efficacy and tolerability compared with risperidone (Potkin et al; ACNP 2005). It also has a unique receptor binding profile, with high affinity for serotonin, noradrenaline and dopamine (DA) receptor subtypes (Shahid et al; ACNP 2005).
Methods: In the current study, we explored the mechanisms of action of asenapine by examining the long-term effects of multiple doses of asenapine on different DA receptors in adult rat brain. Four groups of rats received subcutaneous injections (twice/day) of either vehicle or one of three selected doses (0.03 mg/kg, 0.1 mg/kg or 0.3 mg/kg) of asenapine for 4 weeks. At the end of treatment, animals were sacrificed and their brains were processed for DA receptor autoradiography.
Results: At 0.03 mg/kg, asenapine selectively increased D2 receptor binding in hippocampus (HIP) by 32% ($p<0.05$). A dose of 0.1 mg/kg increased D2 receptor binding in medial prefrontal cortex (mPFC) by 43% ($p<0.05$) and in the HIP by 45% ($p<0.05$), and increased D4 receptor binding in the nucleus accumbens (NAc) by 36% ($p<0.05$), in the caudate-putamen (CPu) by 28% ($p<0.05$), and in the HIP by 48% ($p<0.05$). The highest dose (0.3 mg/kg) increased D1 receptor binding in the mPFC by 26% ($p<0.05$), in the NAc by 59% ($p<0.05$) and in the CPu by 55% ($p<0.05$). Asenapine 0.3 mg/kg also increased D2 binding in the mPFC by 55% ($p<0.05$), in the NAc by 32% ($p<0.05$), in the CPu by 41% ($p<0.05$) and in the HIP by 63% ($p<0.05$), and profoundly elevated D4 receptor binding in the NAc by 71% ($p<0.05$), in the CPu by 70% ($p<0.05$) and in the HIP by 77% ($p<0.05$). No changes in D3 receptor binding were detected with the 3 doses of asenapine used in this study in any of the brain regions examined.
Discussion: These findings indicate that the three doses of asenapine exert differential dose-dependent effects on DA receptors in different brain regions. Asenapine shows preferential effects in the HIP and mPFC and is able to selectively upregulate corticolimbic D2 and striatolimbic D4 receptors without affecting striatal D2 receptors. These unique long-term molecular effects on DA receptors together with effects on other monoaminergic receptors may contribute towards the superior efficacy and tolerability profile demonstrated by asenapine in patients with schizophrenia.

129. A Double-Blind, Placebo-Controlled Study of Olanzapine in Adolescents with Schizophrenia

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Background: Atypical antipsychotics are commonly prescribed for adolescents with schizophrenia, yet the efficacy and safety of these agents have not been systematically investigated in this population in double-blind, placebo-controlled trials. The primary efficacy and safety data from a recently completed double-blind, placebo-controlled study are presented.
Methods: Adolescents (age 13-17 years) with schizophrenia received either flexible doses of olanzapine (2.5-20mg/day) or placebo in a six-week double-blind, placebo-controlled trial. Last-observation-carried-forward mean changes from baseline to endpoint were assessed from the Anchored Version of the Brief Psychiatric Rating Scale for Adolescents total score(BPRS-C), Clinical Global Impressions Scale-Severity (CGI-S), and Positive and Negative Syndrome Scale (PANSS) total, positive, and negative scores. Response was defined as a ≥30% decrease in the BPRS-C and a CGI Severity ≤3. Laboratory values, vital signs, and adverse events were also recorded.
Results: In total, 107 adolescents with schizophrenia (olanzapine n=72, mean age = 16.1±1.3 years; placebo n=35, mean age = 16.3±1.6 years) were randomized (2:1 ratio). The mean (±S.D.) daily dose of olanzapine was 11.1 (4.0) mg/day. Significantly more olanzapine- than placebo-treated patients completed the trial. Olanzapine-treated patients experienced significant improvements compared with those treated with placebo from baseline to endpoint in BPRS-C (-19.3 vs. -9.1, p=.003), the CGI-S (-1.1 vs. -0.5, p=.004), PANSS Total (-21.3 vs. -8.6, p=.005), and PANSS Positive scores (-6.5 vs. -2.7, p=.002). There was a non-significant improvement on the PANSS Negative scale in olanzapine- compared with placebo-treated patients (-3.8 vs. -1.8, p=.081). The treatment response rate was not significantly different between olanzapine- (n=27/72, 37.5%) and placebo-treated patients

(n=9/35, 25.7%, p=.278). Treatment-emergent adverse events occurring significantly more often in olanzapine- versus placebo-treated patients included increased weight and somnolence. Olanzapine-treated patients (4.3±3.3kg) gained significantly more weight than those treated with placebo (0.1±2.8kg, p<.001). Significantly more olanzapine-treated patients experienced treatment-emergent high AST/SGOT, ALT/SGPT, and prolactin, and low bilirubin and hematocrit at any time during treatment. There were no significant differences in the incidence of treatment emergent significant changes from baseline in fasting glucose, cholesterol, or triglycerides at anytime during treatment, although the numeric incidence of normal-to-high triglycerides was approximately three-and-one-half times higher in olanzapine- compared with placebo-treated patients. There were no significant differences between the treatment groups on any of the extrapyramidal symptom scales. Other safety information, including laboratory values, vital signs, and electrocardiographic results, will be presented.

Discussion: In adolescents, olanzapine treatment led to significant improvements on several efficacy measures. The types of adverse events affecting adolescents appear to be similar to those seen in adults.

130. COMT Inhibition Enhances Neuregulin-Induced Cell Migration in B Lymphoblasts

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Background: The catechol-o-methyltransferase (COMT) enzyme metabolises catechol substrates and the gene contains a polymorphism (Val^{158}Met) that influences the activity of the enzyme and is associated with schizophrenia[1]. The COMT Val^{158}Met polymorphism has also been linked with metastasis of human breast cancer[2], hinting that it may play a role in cell migratory processes. It was recently demonstrated that there is a link between this polymorphism and chemotaxis to the product of another schizophrenia susceptibility gene, neuregulin (NRG), in B lymphoblasts: the low activity Met^{158} allele was associated with significantly lower NRG1α-induced chemotaxis[3]. Therefore, this study investigated the relationship between COMT activity and NRG1α-induced chemotaxis in B lymphoblasts from control subjects, selected on the basis of homozygosity at the Val^{158}Met locus, using the selective COMT inhibitor tolcapone.

Methods: Pilot studies investigated the dose response curve of tolcapone on COMT activity in lymphoblast cultures[4], and on NRG1α-induced chemotaxis (n=6 per genotype group). The main study investigated the effect of a single dose of tolcapone (600pM) on NRG1α-induced chemotaxis in 20 cell lines (n=10 per Val^{158}Met homozygote group). Chemotaxis was assayed using either the QCM^{TM} Chemotaxis 5 m 96-well Cell Migration assay kit (Chemicon) or the Innocyte^{TM} Cell Migration assay kit (Calbiochem), according to the manufacturers' instructions.

Results: COMT inhibition was detectable from concentrations of approximately 400pM tolcapone, and maximal COMT inhibition was elicited by 10nM tolcapone. Within this range, there was an inverted-U shaped relationship between tolcapone dose and NRG1α-induced chemotaxis (main effect of tolcapone: p<0.01). Thus, increasing tolcapone produced an increasing potentiation of chemotaxis, with maximal enhancement occurring at 600pM tolcapone. Beyond this dose the potentiation of NRG1α's effect on chemotaxis diminished, and high doses of tolcapone (>1nM) inhibited NRG1 -induced chemotaxis. Furthermore, 600pM tolcapone significantly enhanced NRG1 -induced chemotaxis in 20 lymphoblast cultures from control subjects (p<0.001), although there was no significant effect of Val^{158}Met genotype on this measure (p>0.1).

Discussion: Thus, we confirmed a biological interaction between the schizophrenia susceptibility genes COMT and NRG, identifying a potential novel function for COMT in cell migration. The relationship between COMT activity and NRG1α-induced chemotaxis is complex, with intermediate doses of COMT inhibition potentiating the effect of NRG1α; however, these data are in line with those linking the low activity COMT Met^{158} allele with enhanced NRG1α-induced chemotaxis, since the Met^{158} allele results in reduced, but not abolished, COMT activity. Studies are currently underway to investigate the specificity of this interaction and its molecular basis. However, these findings are, to our knowledge, the first demonstration of a biological interaction between two previously unrelated schizophrenia genes. Furthermore, these data may implicate COMT in neuronal migration, since the latter involves similar molecular pathways to chemotaxis in B lymphoblasts. The interaction may also have implications for metastasis, as both the COMT Met^{158} allele and NRG1 signalling via ErbB2 receptors are metastatic cancer factors. This research was supported by the National Institute of Mental Health 1. Egan et al (2001). PNAS 98: 6917-22. 2. Matsui et al (2000). Cancer Lett 150: 23-31. 3. Sei et al (2005). Soc Neurosci Abs. 4. Chen et al (2004). Am J Hum Genet 75: 807-821.

131. Chromosome 7q PTC Non-Taster Haplotype is Associated with Schizophrenia

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Background: The prevalence of PTC non-tasters has been shown to be increased among schizophrenia patients and non-ill first-degree family member, indicating that PTC tasting may represent an endophenotypic trait of schizophrenia (Moberg et al., American Journal of Psychiatry, 162:788-90, 2005). The ability to taste PTC is determined in large part by genetic variation at the PTC receptor gene TAS2R38. This gene maps to chromosome 7q, a region that has been linked to both schizophrenia and bipolar illness. To date there have been no family-based or case-control association studies of this gene in schizophrenia. This study examined the genotype of schizophrenia patients at two functional SNPs in TAS2R38, ALA49PRO and ILE296VAL to determine if the high rate of non-tasting in our clinical sample is associated with the previously described non-tasting haplotype. Individual genotypes at these two SNPs, as well as 2-allele haplotype frequencies, were compared with a population- based sample using a case-control design.

Methods: Thirty-two schizophrenia patients (previously assessed for PTC taster status) and 75 control subjects were genotyped for both the ALA49PRO and ILE296VAL polymorphisms using Applied Biosystems Assay-on-Demand SNP genotyping assays as per manufacturers protocol. The control group was a population-based sample ethnically matched to the patients. Despite a mixed ethnicity in both the patient and control groups there were no differences in allele frequency detected across ethnic groups. This allowed the different ethnic groups to be analyzed together. Haplotypes were estimated using the PHASE program. Haplotypes and allele frequencies were compared between groups using chi square contingency analysis.

Results: Haplotype frequencies differed significantly between the schizophrenia and control groups (p<0.04). The patient group had an estimated non-taster (A-I) haplotype frequency of 0.64 compared with 0.53 for controls. All patients who were homozygous for the A-I haplotype were non-tasters by clinical assessment. Genotypes for each polymorphism were in Hardy-Weinberg equilibrium.

Discussion: These results suggest a genetically-based association of PTC-non-tasting status with schizophrenia, and provide support to previous linkage findings for schizophrenia on chromosome 7q. Studies to characterize these SNPs in both a case control and family based sample are ongoing. Further studies will be necessary to both confirm this association, and to dissect the neurogenetics underlying it.

132. The COMT Val^{158}Met Polymorphism and Cognition in Schizophrenia

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Sponsor: Travel Awardee, Young Investigator Memorial, 2005

Background: Compelling new data suggests that a single nucleotide polymorphism (Val^{158}Met) of the catechol-O-methyltransferase (COMT) gene on chromosome 22 is implicated in the frontally-mediated cognitive impairments of schizophrenia. The COMT Met allele decreases DA catabolism, thereby increasing the availability of prefrontal DA, whereas the high-activity Val allele increases the breakdown of DA and decreases its prefrontal availability. Previous studies have found that the Met allele is associated in a dose-response fashion with better performance on neuropsychological tests measuring executive functioning, processing speed, and working memory. Although most studies have found that the Met allele is associated with better executive functioning, one study demonstrated that Met homozygotes had better rule learning, but actually had a disadvantage in cognitive flexibility or shifting, suggesting that the relationship between COMT and cognition is more complex than it first appeared. COMT effects on cognition might be explained by the distinction between tonic and phasic DA transmission, with the Met allele increasing tonic DA and decreasing phasic DA, leading to benefits in stability of thinking and sustained cognitive activities, but decrements in cognitive flexibility (e.g., switching tasks). Conversely, the Val allele, with decreased tonic DA and increased phasic DA, might result in benefits on tasks requiring cognitive flexibility or switching, but decrements in cognitive stability (e.g., distractibility). We aimed to further characterize the cognitive patterns associated with the Val and Met alleles, using a comprehensive neuropsychological battery. We hypothesized that the COMT Met allele would be associated with better performance on tests of concept formation, fluency/productivity, and planning, whereas the COMT Val allele would be associated with better performance on tests of cognitive flexibility (e.g., switching) and inhibitory control.
Methods: Participants included 38 outpatients with schizophrenia or schizoaffective disorder (58% men; 76% Caucasian; mean age = 51; mean years of education = 13). Participants were recruited from two parent studies of psychosocial treatments for schizophrenia and underwent a blood draw and comprehensive neuropsychological testing in the domains of Attention, Information Processing Speed, Language, Working Memory, Verbal and Visual Learning and Memory, and Executive Functioning. The executive functioning tests included measures of concept formation, fluency/productivity, planning, and flexibility/shifting. The Positive and Negative Syndrome Scale (PANSS) measured severity of psychiatric symptoms. Participants with the Val/Val (n=9), Val/Met (n=19) and Met/Met (n=10) did not differ on age, gender, education level, diagnosis, age of onset of psychosis, or type of antipsychotic medication (typicals, atypicals, both, or none). Caucasians were underrepresented among Val homozygotes and overrepresented among Met homozygotes.
Results: Val and Met homozygotes performed better than did heterozygotes on a speeded visual scanning test (F=3.3, p=.048). Compared with Val homozygotes only, Met homozygotes had faster reaction times on a sustained attention test (Continuous Performance Test F=12.8, p=.003) and had a trend toward less severe psychiatric symptoms (PANSS Total Score F=4.15, p=.059).
Discussion: These results suggest that the Met allele may be associated with faster information processing speed. The vast majority of between-group differences did not reach statistical significance. Given that the Val^{158}Met polymorphism typically explains a small amount of variance in cognitive performance and given the small size of the current sample, these results need to be replicated with a larger sample.

133. Identification of an Interaction Between the Dopamine D1 Receptor and Niemann-Pick C1 Protein

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Sponsor: Mark von Zastrow

Background: A large body of evidence points to the involvement of dopamine D1 receptors (D1R) in the neurocognitive deficits found in Schizophrenia. A number of investigators have also shown that there is dysregulation of D1R number in the frontal cortex of schizophrenic patients. Recent work has identified a number of Dopamine receptor interacting proteins (DRIPs) that are involved in D1R regulation. Some of these DRIPs are upregulated in schizophrenia. Thus, understanding the regulation of dopamine receptors by interacting proteins is of clinical relevance in that it may be important for the correct functioning of the dopaminergic system whose dysfunction leads to a number of neuropsychiatric diseases.
Methods: In order to identify proteins that interact with the D1R we undertook a yeast two-hybrid screen using the cytoplasmic tail of the Dopamine D1 receptor as bait and a human cDNA brain library as prey.
Results: One positive clone encoded NPC1, a protein involved in the human genetic disease Niemann-Pick Type C (NPC), a neurodegenerative disorder. Interestingly, mild cases of this disorder can present initially with only psychiatric symptoms such as paranoid ideation and auditory hallucinations.
Discussion: Our aims are to biochemically identify the interacting domains of NPC1 and the Dopamine D1 receptor and study the functional consequences of this interaction on D1 receptor activity using the well-established HEK-293 cell heterologous expression system and primary neuronal cultures. These studies will provide fundamental insight into a novel mechanism of dopamine receptor regulation and will also provide an important insight into the psychiatric manifestations present in the hereditary disorder NPC.

134. Are Self-Reports and Physician Impressions of Adherence to Oral Antipsychotics Related to Objective Measures?

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Sponsor: Nina Schooler

Background: The most common ways of assessing adherence to oral antipsychotic medications in research and in clinical practice are self-report and physician/treatment provider report. In a prospective study, we examined the agreement among measures of adherence to oral antipsychotic medications in 50 outpatients with schizophrenia (DSM-IV) at two former Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and Texas Medication Algorithm (TMAP) sites in Texas.
Methods: Subjects were assessed at baseline during a visit to their outpatient clinic and followed for 12 weeks. Adherence was assessed using self-report, physician-report, in home pill counts and electronic monitoring (Medication Event Monitoring (MEMS) downloaded onto laptops at home visits). Adherence was examined as an ordinal variable and as a dichotomous variable (patients taking 80% or more of medication as prescribed were identified as adherent).
Results: Data from pill counts and from electronic monitoring were strongly correlated whether medication adherence was assessed as an ordinal or dichotomous variable (r=.69; p<.0001; Fisher's exact test p<.0005). Self-report of adherence was not significantly related or was only related weakly to objective measures and only when variables were ordinal rather than dichotomous. The physicians' ratings of compliance were significantly correlated with objective measures (r= .35 - .44; p<.01) when considering compliance as an ordinal variable. However, physicians were unable to distinguish adherent from

non-adherent patients when subjects were dichotomized (Fisher's exact test; p <.74).
Discussion: Patients are not good reporters of the amount of medication taken as prescribed. While physicians may be somewhat correct about which patients take more or less medication, they are not able to identify patients that are taking the medication as prescribed. This situation makes it very unclear when to adjust dosing, when to add concomitant medication, and when to switch medications for lack of efficacy. Patients may be labeled as treatment resistant because they are reporting good adherence when they are not taking medication, or medication may not be adjusted appropriately because the physicians' impression is that the patient is not taking the medication as prescribed. Inaccurate assessment of medication adherence represents a significant public health problem that must be addressed.

135. Neuroscience-Guided Cognitive Training in Schizophrenia: Preliminary Data

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Sponsor: William Byerley

Background: The brain plasticity mechanisms that underlie skill learning require key neurobiological "active ingredients" in order to drive cortical representational change, to induce more efficient perceptual processing, and to improve attentional and associative memory functions. We are investigating such a neuroscience-guided approach to the remediation of cognitive deficits in schizophrenia, using a computer-based intensive neuroadaptive training program that heavily engages attentional and reward systems in the brain and that makes use of exercises which, in animals, induce generalized cortical neuroplasticity.
Methods: Fourteen schizophrenic subjects were studied: 7 who participated in our targeted computer-based cognitive training (TCT) program, and 7 who participated in a control condition of graphically interesting computer games (CG). Both groups received 40 hours of intervention (one hour per day, 5 days per week, for 8 consecutive weeks). Both CG control subjects and TCT subjects believed they were receiving the active treatment and kept daily ratings of their level of enjoyment (from 1-7; average CG group rating = 5.65; average TCT group rating = 5.41). All subjects received clinical and neurocognitive assessments at study entry and after the 40 hours of intervention. A pilot subsample also participated in magnetoencephalography (MEG) and functional magnetic resonance imaging (fMRI) studies before and after intervention. The TCT exercises were designed to adaptively change the temporal integration rates for acoustic cues in speech and non-speech stimuli, to explicitly train phonological discrimination and language comprehension, and to focus heavily on auditory temporal processing, attention, working memory, and processing efficiency. The exercises were adaptive in that the stimulus sets of trials were controlled by each subject's trial-by-trial performance and gradually advanced in difficulty as the subject progressed in training. Each trial maintained close attentional control and each correct trial was heavily rewarded. In the CG control condition, subjects played 4 commercially available graphically interesting computer games (CG) per day, 15 minutes per game, analagous to the procedures employed in the TCT exercises.
Results: Analysis of z-score differences (post - pre training) in this preliminary sample showed a trend towards significant improvement in Trails B and in Tower of London measures (p's < 0.09) in the TCT group compared to the CG group, with an effect size of ~1.0 for each of these measures. Qualitatively, pilot imaging data revealed: 1) Plastic changes in primary auditory cortex, as assessed by MEG, in response to successive syllables in background noise in 2 TCT subjects, but not in a CG subject; 2) Plasticity in the response of frontal association cortex in a TCT subject when comparing gamma band desynchronization (magnetic source localization) for a high-load minus a low-load working memory condition before training to that obtained after training; 3) "Normalization" of frontal cortical activation patterns obtained during fMRI of a verbal memory task in 2 TCT subjects after training compared to before training.
Discussion: These preliminary data suggest that, in chronically ill schizophrenic subjects: 1) Computerized intensive neuroadaptive training can successfully drive positive behavioral changes in cognitive function after 40 hours of intervention, an effect not seen in control subjects; 2) Improvements in auditory processing efficiency and working memory, areas trained by the TCT exercises, generalize to non-trained functions, such as executive problem-solving; 3) After training, both primary auditory cortex and prefrontal association cortex demonstrate plasticity in their response to a range of non-trained cognitive tasks.

136. Treatment of Cardiac Risk Factors in Patients with Schizophrenia and Diabetes

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Background: To examine the appropriateness and effectiveness of the outpatient medical management of cardiac risk factors in diabetic patients diagnosed with schizophrenia.
Methods: A cross-sectional analysis of 4236 patients with diabetes treated in five academic internal medicine practices was used. Patients with schizophrenia or a schizophrenia-related syndrome (N=214) were identified based on ICD-9 billing codes. Measures of treatment appropriateness and effectiveness for the management of cardiac risk factors (blood sugar control, blood pressure control, and lipid control) were assessed for this group and a comparison cohort of diabetic patients without severe mental illness (N=3594). Results are reported in terms of odds ratios, adjusted for between-group differences in gender, race, age, and clinic setting.
Results: There were no statistically significant between-group differences on any of the five measures of treatment appropriateness, indicating that patients with schizophrenia received a similar regimen of medical treatment for cardiac risk factors. Despite this, there were significant differences in two of the seven measures of treatment effectiveness, with fewer patients with schizophrenia meeting the clinical quality benchmarks for cholesterol and LDL control. This disparity in effectiveness was not due to failure to provide appropriate medical care, but may be related to the type of treatment provided and/or patient-related variables such as medication adherence and lifestyle factors.
Discussion: Despite appropriate medical management, effective lipid control may be more difficult to attain in at least some patients with schizophrenia. Given the high rates of cardiovascular mortality in this population, additional research to better understand the barriers to effective lipid management is essential.

137. Aripiprazole Effects in Schizophrenia Patients with High or Low Agitation

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Sponsor: James Appel

Background: Patients experiencing acute exacerbation of schizophrenia symptoms including agitation are often judged to be more symptomatic than non-agitated acute patients. It is nevertheless unclear if the presence of baseline agitation has a moderating effect on symptom improvement following antipsychotic treatment. We analyzed data from pivotal clinical trials to evaluate whether patients demonstrated significant symptom improvement when treated with aripiprazole regardless of their baseline agitation level.

Methods: Data were pooled from the first four weeks of four randomized double blind aripiprazole clinical trials of patients diagnosed with acute schizophrenia and randomized to either 10-30 mg/d aripiprazole (n = 785) or placebo (n = 296). For the purpose of this analysis, patients were subdivided into high agitation and low agitation groups according to a baseline PANSS Excited Component (PEC) score of > 14 and also a score of > 4 on a least one PEC item (excitement, hostility, tension, uncooperative, poor impulse control). Separate analyses were performed on three dependent measures: change in PANSS total score, change in Clinical Global Impressions-Improvement score (CGI-I), and change in PEC score. ANOVA was used to evaluate differences among groups (aripiprazole-high agitation (AH), aripiprazole-low agitation (AL), placebo-high agitation (PH), placebo-low agitation (PL)) for each dependent measure. Additionally, because highly agitated patients had higher baseline symptoms, analyses adjusting for baseline PANSS total, CGI-I, and PEC scores were performed.
Results: Aripiprazole treated patients both with high and low agitation scores showed significant decreases at endpoint compared to placebo patients on PANSS total (mean difference: AH vs. PH = -10.7; AL vs. PL = -8.7; $p < 0.05$ for both), CGI-I (mean difference: AH vs. PH = -0.62; AL vs. PL = -0.58; $p < 0.05$ for both), and PEC (mean difference: AH vs. PH = -2.5; AL vs. PL = -1.8; $p < 0.05$ for both). These differences remained significant after adjusting for baseline score on each dependent measure ($p < 0.0001$ for PANSS total, CGI-I, PEC).
Discussion: Acute schizophrenia patients treated with aripiprazole in both the high and low agitation groups showed significant improvement in overall symptoms, clinical impressions, and agitation compared to placebo-treated patients. The effects remained when the dependent measures were adjusted to normalize baseline scores. This suggests that patients with acute schizophrenia benefited from aripiprazole treatment regardless of baseline agitation level.

138. Anterior Cingulate Bundle Abnormality in Children and Adolescents with Schizophrenia

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Background: Schizophrenia is associated with a constellation of clinical and cognitive symptoms. One hypothesis that unifies the diversity of symptoms involves the disruption of connectivity between brain regions. As white matter provides rapid and efficient communication between brain regions, this study was initiated to assess the early disruption of white matter pathways in children and adolescent with schizophrenia.
Methods: Diffusion Tensor Images (DTI) were obtained in twelve directions on 14 children and adolescents with schizophrenia and 14 age and gender matched control. The DTI images were acquired in twelve directions on a 3 Tesla Siemen's TRIO scanner and transformed into fractional anisotropy (FA) images. A 12-parameter affine transformation was applied to the FA images using SPM99 to render the images into a common stereotactic space. The images were subsequently smoothed with a 5 mm FWHM Gaussian filter prior to a cluster-wise group analysis.
Results: Children and adolescent patients with schizophrenia demonstrated a significant decrease in FA in the right anterior cingulate bundle ($p<0.05$, Bonferroni corrected on the cluster-level).
Discussion: The voxel based approach coupled with DTI offers the advantage of evaluating neural tracts within the brain without a priori assumptions. Utilizing this approach, children and adolescents with schizophrenia demonstrated a significantly reduced FA in the anterior cingulate bundle. The role of the anterior cingulate and its disruption early in the course of schizophrenia will be discussed.

139. Altered Cerebral Processing of Reward Uncertainty in Patients with Schizophrenia While Medication-Free and on Atypical Neuroleptics

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Background: There is considerable evidence that the pathophysiology of schizophrenia involves dopamine dysregulation in fronto-striatal and -midbrain circuits, likely impacting on the neural substrates of reward processing and contributing to core schizophrenic symptoms such as anhedonia, amotivation, impulsivity, and substance use. Investigation of this pathophysiology in vivo has been limited by the confound of antipsychotic treatment in most patients, and by the absence of incisive neuroimaging approaches. We studied patients after 3 weeks medication-free (OFF) and then after 3 weeks ON atypical antipsychotics with a novel event-related fMRI paradigm based on the findings of Schultz *et al* (Science 2003) that midbrain dopaminergic neurons in primates code for discrete statistical properties of expected reward, exhibiting phasic responses that code reward prediction error, and sustained responses that are highest with maximal reward uncertainty (probability=50%). This latter activity is also reflected by increased signaling in dopaminergic forebrain regions, such as striatum, anterior cingulate, and prefrontal cortex (PFC).
Methods: 9 patients with schizophrenia and 22 healthy individuals matched for age (p=0.22) and sex ratio were scanned in an event-related 3T-fMRI paradigm during presentation of four simulated slot machines that systematically varied reward probability(P) and magnitude(M): M=0/P=0,M=10/P=0.50,M=20/P=0.25,or M=20/P=0.50. These specific combinations allowed for unambiguous parsing of the effects of P and M. Before testing, subjects were trained to know P and M of each stimulus and were told that they would receive a portion of the winnings displayed. At the start of each trial, a pseudorandomly selected slot machine was displayed to the participant (S1: 1 sec). After a 14-sec delay, during which P and M were continually displayed, the subject learned the outcome of the trial (S2: 1 sec). There were 96 trials per session. Data were processed in SPM99 (random effects threshold, p =0.05) using a general linear model with separate regressors for the phasic responses at S1 and S2 and the sustained response during the delay.
Results: are given for the sustained reward response during the delay, as P increased from 0.25 to 0.50 (maximal uncertainty) with M constant. **CONTROL *vs* OFF:** Controls showed greater BOLD signal increases in bilateral caudate, bilateral basal forebrain (BA 47), R lentiform nucleus, and L medial PFC. The OFF group had greater activation in R dorsolateral PFC (BA 9) and L dorsomedial PFC (BA 8). **CONTROL *vs* ON:** Controls had more activation in L dorsolateral PFC (BA 8/9). ON showed increased activation in L caudate, bilateral dorsomedial PFC (BA 6,9), and bilateral parietal lobe (BA 24). **ON *vs* OFF:** When patients were ON, they activated bilateral striatum, bilateral temporal cortex (BA 21), L midbrain, and R cingulate more than when they were OFF. While OFF, they showed increased signal in bilateral superior temporal gyrus (BA 21,22), L cingulate (BA 32), and L parietal lobe (BA 7)
Discussion: This event-related fMRI study demonstrates atypical sustained brain activity during reward anticipation in patients with schizophrenia. With increasing uncertainty of reward, OFF subjects did not show the same degree of increased activity in striatum and basal forebrain as did controls. When ON, patients had increased activation of reward centers. These findings suggest that processing of reward uncertainty, a critical function of mesolimbic and mesocortical tracts, is dysfunctional in schizophrenia. The findings also imply that atypical antipsychotics alter neurotransmission in reward circuits.

140. Asenapine: A Novel Psychotherapeutic Agent with a Unique Human Receptor Binding Signature

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Sponsor: Stephen Arneric

Background: Asenapine is believed to offer an advance in the therapeutic management of schizophrenia and bipolar mania based on promising clinical trial results (Potkin et al; ACNP 2005). The mechanism of action of asenapine was explored by comparing its human receptor binding properties with other antipsychotic drugs.
Methods: Objective comparison of all drugs was achieved under comparable binding assay conditions using membranes from cells expressing cloned human serotonin (5HT1A, 5HT1B, 5HT2A, 5HT2B, 5HT2C, 5HT5, 5HT6, 5HT7), norepinephrine (α1, α2A, α2B, α2C), dopamine (D1, D2S, D2L, D3, D4), histamine (H1, H2, H3) and acetylcholine (M1, M2, M3, M4) receptors. Asenapine was also tested for general receptor specificity profiling in a panel of 90 different binding assays including enzyme, transporter and ion channel molecular targets.
Results: Asenapine showed no significant activity in the general receptor specificity profiling. Asenapine exhibits a unique human receptor binding signature based on a number of observations: i) the rank order of receptor affinity for asenapine is distinct from olanzapine, risperidone, quetiapine, aripiprazole, ziprasidone, clozapine and haloperidol , ii) compared with the other drugs tested, asenapine exhibits a stronger serotonergic component with subnanomolar affinity (Ki in nM) for 5HT2C (0.03), 5HT2A (0.07), 5HT2B (0.18), 5HT7 (0.11), and 5HT6 (0.25) receptors, with reference to D2 (1.3) receptors, iii) in contrast to the other drugs, asenapine is likely to have strong interaction with an additional ensemble of therapeutically relevant receptors: 5HT7, 5HT6, 5HT5 (1.6), D3 (0.42) receptors and α1 (1.20), α2 (0.33-1.2) adrenoceptors, at equivalent therapeutic doses, and iv) unlike olanzapine, clozapine and quetiapine, asenapine has a lower affinity for both histamine and muscarinic receptors when compared to its D2 receptor affinity.
Discussion: Overall the human receptor binding profile of asenapine differs significantly from that of other antipsychotic drugs. Such differentiation in receptor pharmacology may contribute towards its novel clinical profile, in particular with respect to an impact on negative and cognitive symptoms. Asenapine might serve as a novel psychotherapeutic agent to test a multi-target approach towards the effective and safe treatment of schizophrenia and bipolar disorder.

141. Variation in Catecol-O-Methyltransferase and GRM3 on Working Memory Load and Prefrontal Cortical Physiology

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Background: The Catecol-O-methyltransferase (COMT) Val158Met polymorphism accounts for differential enzyme activity and dopamine catabolism in the prefrontal cortex (PFC). The high-activity Val allele has been associated with risk for schizophrenia, and inefficient increase in PFC blood-oxygen-level-dependent (BOLD) activation during working memory (WM) tasks. GRM3, a metabotropic glutamate receptor that modulates synaptic glutamate, has also been associated with schizophrenia, with the A rather than G allele of single-nucleotide polymorphism 4 (hCV11245618) related to physiological inefficiency, and reduced glial glutamate transporter levels in the PFC. Further, some schizophrenia patients, and subjects exposed to NMDA-receptor antagonists, were noted to have greater prefrontal BOLD activation with WM load. We therefore examined the additive effects of these genes on incremental WM load and PFC BOLD activation.
Methods: Twenty-nine healthy subjects (10 COMT Val and GRM A homozygotes, 6 COMT Val homozygotes and GRM G carriers, 6 COMT Met and GRM A homozygotes, and 7 COMT Met homozygotes and GRM G carriers) were studied with fMRI while performing 1-back and 2-back WM tasks.
Results: The 4 groups were matched for demographic characteristics and n-back performance. Load effects (2-back>1-back) were identified at the bilateral dorsal and ventral PFC. The functional region-of-interest at the right dorsal PFC had COMT effects with greater (inefficient) activation in Val relative to Met homozygotes ($F(1,27)=4.50$, $p=0.043$). Further, GRM A homozygosity interacted to give disproportionately greater load-dependent dorsal PFC activation in COMT Val homozygotes, but not in Met homozygotes (load-by-COMT-by-GRM interaction, $F(1,25)=4.42$, $p=0.046$).
Discussion: Relatively deleterious dopaminergic and glutaminergic alleles may interact to influence BOLD activation to incremental WM load in the dorsal PFC, and be of potential significance in the neuropharmacology of schizophrenia.

142. Cortisol and Cytokines in Chronic and Treatment-Resistant Patients with Schizophrenia: Association with Psychopathology and Response to Antipsychotics

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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: The bilateral communication between the immune and neuroendocrine systems plays an essential role in modulating the adequate response of the hypothalamic-pituitary-adrenal (HPA) axis to the stimulatory influence of cytokines and stress-related mediators. Growing evidence suggests that neuro-immune-endocrine cross-talk may be impaired in schizophrenia.
Methods: We determined the relationship between cortisol, cytokines interleukin-2 (IL-2) and interleukin-6 (IL-6) and symptoms in schizophrenia during treatment with typical and atypical antipsychotic drugs. Subjects included 30 healthy controls (HC) and 78 schizophrenic (SCH) inpatients. SCH were randomly assigned to 12-week treatment with 6mg/day of risperidone or 20mg/day of haloperidol using a double-blind design. Clinical efficacy was determined using the Positive and Negative Syndrome Scale (PANSS). Serum cortisol and IL-2 levels were assayed by radioimmunometric assay, and serum IL-6 levels by quantitative enzyme-linked immunosorbent assay.
Results: Following a 2-week washout period, serum levels of cortisol, IL-2 and IL-6 were increased in patients with schizophrenia compared to HC. Elevations in cortisol were associated with increase in both IL-2 and IL-6 in schizophrenia. Moreover, elevations in cortisol were associated with negative symptoms and IL-2 with positive symptoms. Twelve weeks of risperidone treatment significantly decreased elevated cortisol and improved negative symptoms, but produced similar effects on IL-2 and IL-6 as well as on positive symptoms compared to haloperidol. The improvement of negative symptoms was related to the change in cortisol.
Discussion: Our results suggest the imbalance in the HPA axis and cytokine system in patients with SCH is implicated in clinical symptoms, and is improved with atypical antipsychotic treatment.

143. BDNF Levels and Genotype Are Associated with Antipsychotic-Induced Weight Gain in Patients with Chronic Schizophrenia

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Sponsor: Travel Awardee, sanofi-aventis, 2005

Background: Several lines of recent evidence indicate an involvement of brain-derived neurotrophic factor (BDNF) in the regulation of eating behavior and body weight, as well as in antipsychotic action in the

central nervous system. A valine (val) to methionine (met) substitution in the 5' pro-region of the human BDNF protein has been identified recently. Therefore, the aim of the present study was to investigate the significance of this type of polymorphism in antipsychotic-induced body weight gain in chronic patients with schizophrenia.
Methods: The Val66Met polymorphism was genotyped in 210 Chinese Han patients with chronic schizophrenia. Using enzyme immunoassay, serum BDNF was measured in 196 schizophrenia patients chronically treated with antipsychotics. Mean weight change was evaluated retrospectively by means of clinical records and the weight at the time of the visit.
Results: The patients with the 66Met variant allele showed significantly greater weight gain than those without this allele. The effect was strongest in the male patients and not apparent in the female patients. Furthermore, the Val66Met polymorphism of the BDNF promoter region affects the serum BDNF levels in female patients with chronic schizophrenia, showing that serum BDNF levels were significantly lower in patients with the 66Met variant allele than those without this allele. Additionally, there is a significantly negative correlation between serum BDNF levels and weight gain in schizophrenic patients, especially in female subjects.
Discussion: These findings suggest that genetically determined interindividual differences in BDNF may influence weight gain induced by antipsychotic drugs in schizophrenia.

144. Histone Hyper-Acetylation Enhances Memory Storage and Facilitates Synaptic Plasticity via the Transcription Factor CREB

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Background: Transcriptional activation is thought to be a key process in long-lasting forms of memory and synaptic plasticity. This activation is directed by transcription factors and their coactivators, which regulate gene activation via chromatin remodeling, histone modification and interactions with the basal transcription machinery. One type of histone modification associated with transcriptional activation is acetylation, which is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) that add or remove acetyl groups from histones, respectively. Recently, we have demonstrated that the transcriptional coactivator CREB-binding protein (CBP), a potent HAT, is involved in specific forms of long-term memory and synaptic plasticity.
Methods: To examine the role of histone acetylation in memory, we have examined the effects of the HDAC inhibitor, trichostatin A (TSA) on memory and synaptic plasticity. TSA induces a histone hyper-acetylation state that correlates with increased transcriptional activation.
Results: Behaviorally, intrahippocampal injection of TSA significantly enhanced memory for contextual, but not cued, fear conditioning. Further, hippocampal slices treated with TSA showed significantly enhanced long-term potentiation (LTP). To define the molecular basis of the enhancements observed following TSA treatment, we examined mice with targeted deletions of two CREB isoforms (α and Δ; CREB $\alpha\Delta$ mice). CREB $\alpha\Delta$mice did not show enhanced memory when treated with TSA, and hippocampal slices from CREB $\alpha\Delta$ mice failed to show TSA-enhanced LTP. Additionally, using quantitative real-time RT-PCR, we demonstrated that only a subset of CREB-target genes is affected by TSA during memory consolidation.
Discussion: These experiments underscore the involvement of histone acetylation in synaptic plasticity and memory and begin to define a molecular mechanism by which HDAC inhibitors regulate memory storage, providing insight into their potential use as therapeutic drugs to treat cognitive impairments. Supported by: Neurodegenerative Diseases training grant fellowship to MAW. Merck Foundation, NIH, Packard Foundation, University of Pennsylvania Research Foundation and Whitehall Foundation to TA. The authors affirm that this work has been carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was fully approved by the IACUC of the University of Pennsylvania.

145. Changes in Natural Killer Cell Function, Inflammatory Signaling and Plasma IL-6 Production During Acute Stress in Patients with Major Depression

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Sponsor: Andrew Miller

Background: There has been increasing interest in reciprocal interactions among the nervous, endocrine and immune systems in psychiatric disorders such as major depression. One hypothesis suggests that proinflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor (TNF)-alpha, and their signaling pathways, including nuclear factor- kB (NF-kB) and its downstream mediator, IL-6, act as neuromodulatory factors that contribute to the pathophysiology of major depression. In addition, alterations in other immune parameters, including NK cell numbers and function in major depression, have been described. While activation of both inflammatory mediators and alterations in NK cells have been reliably reported in major depression, few studies have examined the relationship between these immune variables under baseline conditions. Furthermore, few studies have examined the relationship among these immune variables under dynamic conditions (e.g. acute laboratory stress).
Methods: Natural killer cell numbers, NF-kB DNA binding and IL-6 production in the peripheral blood were examined during the Trier Social Stress Test (TSST) (a public speaking and mental arithmetic task) in two groups of patients: patients with DSM IV major depression defined by a SCID diagnosis and non-depressed subjects. Plasma IL-6 was measured with the enzyme-linked immunosorbent assay (ELISA) (R&D systems), natural killer cell numbers were determined using a FITC conjugated monoclonal antibody against CD16/56 markers on PBMC and acquiring the fluorescent intensity on a FACS-Calibur (BD Biosciences), and NF-kB DNA binding was determined using a DNA binding ELISA (Active Motif).
Results: As compared to the control group, patients with major depression (n=11) showed a higher phasic response of NF-kB DNA binding and plasma IL-6 to TSST ($p<.04$) compared to non depressed subjects (n=22). There was no observed difference in NK responsiveness to the TSST in both groups, although both depressed (n=8) and non-depressed subjects (n=18) exhibited a significant increase in NK cell numbers during stress. No correlation between NK cell number and plasma IL-6 or NF-kB DNA binding during the TSST were found. In contrast, maximal TSST-induced NF-kB DNA binding, which occurred relatively early during stressor challenge, was significantly correlated with maximal TSST-induced plasma IL-6 concentrations which occurred relatively late following stress ($r=.45$, $p=.02$).
Discussion: The observed dissociation between natural killer cell numbers and IL-6/NF-kB DNA binding levels during the TSST reflects the specificity of inflammatory changes in major depression, and indicates that changes in NK cell distribution do not account for the changes in NF-kB DNA binding during psychosocial stress. In addition, the data suggest that activation of inflammatory signaling pathways (e.g. NF-kB) may precede induction of downstream mediators such as IL-6. Finally, the data support the notion that patients with major depression may be vulnerable to increased activation of inflammation during stress, thereby providing a potential link between depression and other medical pathologies wherein inflammation serves as a pathophysiologic contributor.

146. The Phosphodiesterase Type IV Inhibitor, Rolipram, Activates Septohippocampal Neurons

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Background: Significant behavioral evidence suggests an important role for the cAMP-specific phosphodiesterase, PDE4, in depression and in learning and memory. Thus, the selective PDE4 inhibitor, rolipram, has antidepressant effects and attenuates scopolamine-induced impairment of working and reference memory in rats (Zhang and O'Donnell, 2000). Rolipram also reverses the amnesic effects of intra-hippocampal infusions of NMDA receptor antagonists and MEK inhibitors and produces a persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model (Gong et al., 2004). Several PDE inhibitors are currently in the pipeline for treatment of various CNS disorders. The cholinergic nucleus of the medial septum/diagonal band of Broca (MSDB) projects to the hippocampus and controls hippocampal BDNF levels, hippocampal theta rhythm and associated learning and memory tasks. The MSDB which has high levels of PDE4 mRNA, degenerates in Alzheimer's disease, Lewy body dementia, Parkinson's disease, alcoholic Korsakoff syndrome and several other neurodegenerative disorders. The MSDB is also a key locus for the memory-impairing effects of scopolamine. The goal of the the present study was to use an electrophyisological approach to study the role of PDE4 in controlling the excitabilty of the septohippocampal neurons located in the MSDB.
Methods: Whole cell recordings were performed on electrophysiologically-characterized septohippocampal GABA-type neurons in a rat brain slice preparation of the MSDB.
Results: In a previous study, we reported that scopolamine reduces the firing rate of septohippocampal GABAergic neurons. In the present study, the selective PDE4 inhibitor, rolipram (1-10 μM, increased the basal firing rate in 5 of the 10 neurons tested. Rolipram also increased the frequency of spontaneously occurring post-synaptic currents (control: 12 ± 4 Hz; rolipram: 18 ± 5 Hz; p=0.02; n=3) in these neurons. Forskolin (10 μM), which increases intracellular cAMP levels by activating adenylate cyclase, dramatically increased both the basal firing rate (control: 42 ± 8.3 Hz; forskolin: 127 ± 17 Hz; n=6) as well as the synaptic frequency (control: 4 ± 1 Hz; forskolin: 8 ± 2 Hz; n=5). Both these effects were potentiated by rolipram (synaptic frequency: forskolin: 8 ± 2 Hz; rolipram forskolin: 15 ± 3 Hz; n=5). In double immunohistochemical studies, PDE4A immunoreactivity colocalized with a vast majority of septohippocampal cholinergic neurons and with a small percentage of septohippocampal GABAergic neurons.
Discussion: Thus, PDE4 plays an important role in cAMP metabolism in septohippocampal GABA-type neurons of the MSDB. These cellular actions may contribute to the reported memory-enhancing and antidepressant effects of rolipram.

147. Creation of Designer Biogenic Amine Receptors via Directed Molecular Evolution

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Sponsor: Bryan Roth

Background: Members of the large biogenic amine family of G-protein Coupled Receptors—GPCRs—are involved in a many processes within the central and peripheral nervous systems and represent significant targets for drug therapy. Current pharmacological and transgenic techniques are insufficient for measuring functions of a single GPCR in a specific cell or tissue in vivo. To circumvent the shortcomings of these traditional approaches, we have developed designer biogenic amine receptors that, like receptors activated solely by a synthetic ligand (RASSL), only respond to a synthetic ligand versus the receptor's native ligand. Unlike RASSLs, however, these designer receptors have the distinction that they are activated by an otherwise innocuous, biologically-inert synthetic drug.
Methods: A directed evolutionary approach in yeast was utilized to generate a designer acetylcholine muscarinic 3 (M3) receptor responsive to clozapine-N-oxide, a biologically-inert metabolite of clozapine, with a concomitant loss in acetylcholine activation. We have also used transgenic mouse technology to specifically express the designer GPCR in a subpopulation of cortical neurons using an inducible promotor.
Results: When the designer M3 receptor is expressed in either HEK-293T cells or primary human pulmonary artery smooth muscle cells (hPASMCs) it can be activated by nanomolar concentrations of clozapine-N-oxide to stimulate normal Gq/11-coupled responses, such as PLC-mediated PIP2 hydrolysis and release of internal calcium stores. Additionally, treatment of hPASMCs expressing the designer M3 receptor with clozapine-N-oxide lead to rapid activation of ERK-1/2, which does not occur in hPASMCs expressing wildtype M3 receptor. Using a bioinformatics approach we have begun to develop similar designer receptors for all five acetylcholine muscarinic receptor family members, including the Gi/o-coupled M2 and M4 receptors. We have also created several lines of transgenic mice which express the designer receptor in discrete neuronal populations using an inducible promotor.
Discussion: Our data demonstrate that a combination of directed evolution and bioinformatics approaches can be used to create a family of designer biogenic amine GPCRs. The ability to express these receptors in a spatio- and temporally-restricted manner opens up a new approach to dissecting the relevance of various signaling cascades for neuronal functioning. Supported by the NIMH Psychoactive Drug Screening Program, RO1MH576345 and KO2MH01366 to BR; BA is supported by NRSA fellowship GM074554-01.

148. Elevated Membrane Cholesterol Limits Metabotropic Glutamate Receptor (mGluR) Suppression of NMDA Function: A Potential Contribution to Antipsychotic Action?

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Sponsor: Daniele Piomelli

Background: Deficient N-methyl-D-aspartate (NMDA) receptor function has been proposed to account for many positive and negative symptoms of schizophrenia. To understand mechanisms of NMDA receptor deregulation, we studied membrane enrichment with cholesterol effects on NMDA receptor function by using group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG)-mediated suppression of NMDA responses as a model.
Methods: Experiments were conducted on organotypic hippocampal cultures prepared from 1-week-old Wistar rats and grown for 2 weeks as described previously (Blaabjerg et al., 2003). Moderate, concentration-dependent excitotoxicity (20-30% of cells killed) induced by 30-min exposure of cultures to NMDA (50 μM) was measured as propidium iodide uptake using confocal microscopy 24 hours after the NMDA exposure, and served as an indicator of NMDA receptor function.
Results: As in our earlier experiments (Blaabjerg et al., 2003), application of the mGluR agonist DHPG (1-100 μM) 2-hours before NMDA markedly reduced NMDA toxicity. To examine a possibility that mGluR-mediated reduction of NMDA toxicity could be dependent on membrane cholesterol contents, we used cholesterol-saturated methyl-β-cyclodextrin (Chol-MβCD, 0.01-0.1 mM). Treatment of cultures with Chol-MβCD was started 1 hour before DHPG and continued throughout DHPG and NMDA exposure. At 0.1 mM it markedly reduced the DHPG effect without affecting NMDA toxicity per se (DHPG+NMDA, 74±3.7%, p<0.001; NMDA only,100±5.0%;

Chol-MβCD+DHPG+NMDA, 106±5.1%, p>0.05; all data normalized to NMDA alone, all experiments were repeated in triplicate on 34-36 cultures, t-test with Bonferroni correction). The cholesterol-enriched medium did not affect the NMDA toxicity level (108±4.3%, p>0.05). Increase in nerve cell membrane cholesterol contents caused by Chol-MβCD treatment was confirmed by measuring membrane cholesterol concentration following Chol-MβCD treatment.
Discussion: We have recently shown that persistent activation of group I mGluRs suppressed NMDA-mediated excitation in a phospholipase C (PLC)-dependent manner, reduced NMDA-mediated membrane currents and facilitated endocytosis protein Rab5b synthesis (Blaabjerg et al., 2003, Arnett et al., 2004). The mechanism of the cholesterol action observed here remains unknown but may represent interference with Rab5b-dependent NMDA receptor endocytosis. Together, these findings suggest that increasing membrane cholesterol could limit group I mGluR-PLC-Rab5b mediated depression of NMDA receptor function and offer a new look at a potential significance of regulation of membrane lipids in ethiopathogenesis and treatment of schizophrenia.

149. Enzymes Coupled with Cyclooxygenase Are Altered in the Brain of Cyclooxygenase-1 Deficient Mice: Implications for Neuroprotection in Neuropsychiatric Disorders
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Sponsor: Past Travel Awardee, Bristol-Myers Squibb, 2003

Background: Currently, there is an intense debate on the potential use of nonsteroidal anti-inflammatory drugs (NSAIDs) in Alzheimer's disease. The effects of NSAIDs are largely related to the inhibition of the enzymatic activity of cyclooxygenase (COX)-1 and -2, key enzymes involved in arachidonic acid metabolism to prostaglandins. Animal studies and preliminary clinical data suggest that selective COX-2 inhibitors also may be beneficial in psychiatric disorders such as schizophrenia or mood disorders. Additionally, the mood stabilizers lithium, valproate, and carbamazepine commonly down-regulate COX-mediated conversion of arachidonic acid to prostaglandin E_2.
Methods: We have recently reported that COX-2 deficient ($COX\text{-}2^{-/-}$) mice show a compensatory increase in brain COX-1 enzyme activity, protein and mRNA levels, as well as changes in upstream and downstream coupled enzymes. To further characterize the interactions between enzymes involved in COX-mediated brain arachidonic acid metabolism and the effects of selective inhibition of the two COX isozymes, we examined enzymatic activity and protein and mRNA expression of brain phospholipase A_2 (PLA_2), COX-2, and prostaglandin E_2 synthases (PGESs) in $COX\text{-}1^{-/-}$ mice.
Results: We found an increase in the enzymatic activity and protein levels of cytosolic $cPLA_2$ and secretory $sPLA_2$, which supply the arachidonic acid substrate to COX, in the $COX\text{-}1^{-/-}$ mice compared with wild-type. Brain prostaglandin E_2 level was significantly increased, whereas thromboxane B_2 level was decreased. These changes were accompanied by an increase in the enzymatic activity and protein levels of COX-2. These data suggest that brain PGE_2 is selectively derived from the COX-2 pathway, whereas TXB_2 is selectively derived from the COX-1 pathway. Consistent with the COX-2 up-regulation, we found an increase in NF-κB activation, which was accompanied by the up-regulation of the phosphorylated IκB and upstream IκB kinase in $COX\text{-}1^{-/-}$ mice. Enzymes downstream from COX were also affected: the protein levels of microsomal PGES-1 and -2, but not of cytosolic PGES, were decreased in $COX\text{-}1^{-/-}$ mice.
Discussion: Taken together, our data suggest that COX-1 deficiency can affect the expression of the reciprocal isozyme, COX-2, and of coupled upstream and downstream PLA_2 and PGES enzymes to overcome defects in brain prostaglandin synthesis. Studying this pathway in animal models of neuropsychiatric disorders will help to elucidate the individual role of COX isozymes in the molecular mechanisms underlying the pathological changes and the potential beneficial effects of early COX inhibition.

150. ERK MAP Kinase Signaling in Postmortem Brain of Suicide Subjects: Differential Regulation of Upstream Raf Kinases Raf-1 and B-Raf
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Background: Mitogen-activated protein/extracellular signal-regulated kinase (ERK) is a family of serine/threonine protein kinases that are critical for several neuronal functions regulated by growth factors/neurotrophins, and their receptors associated with tyrosine kinases. These include neuronal differentiation and survival of neurons during development, as well as survival and adaptive responses of mature neurons including synaptic plasticity and learning and memory. The activation of ERKs involves a cascade of events in which a small GTP binding protein, Ras, recruits Raf kinases to the plasma membrane. Raf kinases then phosphorylate and activate ERK kinase, or MEK, which in turn phosphorylates and activates ERKs. The sequential nature of this cascade thus provides multiple points at which the responses can be regulated by phosphorylation, which allows tremendous amplification of extracellular signals. Previously, we observed that activation of ERK-1/2, the downstream component of ERK signaling, is significantly reduced in postmortem brain of suicide victims. The present study was undertaken to further examine whether suicide brain is also associated with abnormalities in upstream molecules in ERK signaling.
Methods: The study was performed in prefrontal cortex (PFC) and hippocampus obtained from 28 suicide victims and 21 normal controls. Subjects were diagnosed using DSM IV criteria. mRNA levels of Raf-1, B-Raf, and cyclophilin were measured by quantitative RT-PCR. Protein levels of Raf-1 and B-Raf were determined by Western blot, whereas their catalytic activities were determined by immunoprecipitation and enzymatic assays.
Results: It was observed that the catalytic activity of B-Raf was significantly reduced in PFC and hippocampus of suicide subjects. This decrease was associated with a decrease in its protein, but not mRNA, level. On the other hand, catalytic activity, and mRNA and protein levels, of Raf-1 were not altered in postmortem brain of suicide subjects. The observed changes were not related to confounding variables; however, Raf-1 showed a negative correlation with age. Also, the changes in B-Raf were present in all suicide subjects, irrespective of psychiatric diagnosis.
Discussion: Our finding of the selectively decreased activation of B-Raf, but not of Raf-1, in postmortem brain of suicide subjects therefore may be of critical importance and offer a new insight into the pathophysiology of suicide, namely, that B-Raf may be involved in mechanism by which the ERK pathway may be regulated in suicidal behavior.

151. Adderall® Produces Differential Dopamine Overflow When Compared to D-Amphetamine in Rat Striatum
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Sponsor: Greg Gerhardt

Background: Psychostimulants currently in use for the treatment of Attention-Deficit/Hyperactivity Disorder (ADHD) include both single isomer preparations and isomer mixtures. Adderall®contains a novel combination of d- and l- amphetamine, which has demonstrated equal efficacy to d-amphetamine. However, Adderall® shows differential patient responses. We hypothesized that d-amphetamine and Adderall® would differentially affect dopamine (DA) overflow when administered by reverse microdialysis in rat striatum at levels of stimulation relevant for ADHD. Since it is difficult to know exactly

what concentration would be relevant to ADHD for stimulation in the rat brain, we conducted a dose-response study.
Methods: A 2 mm microdialysis probe was inserted into the urethane-anesthetized Fischer 344 rat striatum for perfusion with aCSF or aCSF + drug while dopamine (DA) and its metabolites were determined by HPLC-EC at 20 minute intervals. After establishing a baseline, d-amphetamine or Adderall® was applied for 20 minutes by reverse microdialysis over a large range of concentrations.
Results: DA overflow produced by 0.01 mM or 0.1 mM d-amphetamine (~30 nM DA) was not significantly different. However 0.01 (26.4 nM DA) and 0.1 (50.8 nM DA) mM Adderall® produced significantly different ($p<0.05$) levels of DA.
Discussion: These data support that d-amphetamine and Adderall® differ in their ability to cause DA overflow, which may help explain some of the clinical differences seen in the use of these drugs to treat ADHD.

152. Distinct Roles for MT_1 and MT_2 Melatonin (MLT) Receptors in MLT-Mediated Phase Shifts of Circadian Rhythms

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Sponsor: Margarita Dubocovich

Background: Affective disorders are often associated with desynchronization of endogenous circadian and/or seasonal rhythms. Knowledge about the mechanisms by which endogenous oscillators are synchronized by non-photic stimuli is important for the design of therapeutic strategies for treatment of mood disorders. In humans, physiological doses of the hormone MLT promotes sleep and phase shifts circadian rhythms at dusk or dawn (Arendt and Skene, Sleep Med. Rev. 9:25, 2005; Dubocovich et al., Front. Biosc. 8:d1093, 2003). MLT mediates its effects through activation of two G-protein coupled receptors, the MT_1 and MT_2 (Dubocovich et al., Front. Biosc., 2003).
Methods: This study determined the role of MT_2 MLT receptor activation on MLT-mediated phase shifts of spontaneous circadian rhythms of neuronal firing rate in the suprachiasmatic nucleus (SCN) brain slice and of circadian rhythms of wheel running activity in vivo using wild type (WT) C3H/HeN mice and mice with genetic deletion of either the MT_1 (MT_1KO) or MT_2 (MT_2KO) MLT receptors. Spontaneous circadian rhythms of neuronal firing rate were recorded from SCN coronal brain slices (400 μm) using the single-unit recording technique. CT12 (Circadian Time) is the onset of activity for host mice.
Results: The effect of MT_1 and MT_2 MLT receptor activation on circadian rhythms was assessed by determining the shift of onset of wheel-running activity rhythms in mice kept in constant dark. SCN brain slices from WT mice showed a peak of neuronal firing at CT 6.1 ± 0.1 (n=5). Micro-drop application of MLT (10 pM for 10 min) to the SCN brain slice at CT10 phase advanced the peak to CT 3.4 ± 0.2 (n=4) ($p < 0.001$) and at CT2 phase delayed the peak to CT 7.2 ± 0.1 (n=6) ($p < 0.001$) as compared to vehicle treated slices (CT 6.1 ± 0.1, n=6). MLT applied at CT10 induced phase advances of identical magnitude in the SCN brain slices from MT_1KO mice but did not affect clock phase in slices from MT_2KO mice (CT 5.9 ± 0.3, n=5) as compared to vehicle treated (CT 6.1 ± 0.1, n=4). These results demonstrate that in the SCN brain slices MLT phase-shift neuronal firing rhythms by activation of MT_2 MLT receptors. Administration of the MLT agonist 6-chloroMLT (6Cl-MLT: 90 μg/mouse, sc), which has higher affinity for the MT_2 than the MT_1 receptor, to WT mice at CT10 advanced (1.44 ± 0.11h, n=18 , $p <0.001$) and at CT2 delayed (0.78 ± 0.07 h, n=12, $p <0.001$) the onset of wheel running activity rhythms as compared to vehicle treated mice (CT10: 0.1 ± 0.07h, n=14; CT2: -0.2 ± 0.06h, n= 10). Paradoxically, 6Cl-MLT given at either CT 10 or CT2 and MLT given at CT10 to MT_1KO mice did not phase shift the onset of circadian rhythms of wheel running activity. However, MLT administration at CT10, significantly phase advanced wheel running activity rhythms in the MT_2 KO mice (1.3 ± 0.18h, n=9, $p<0.001$) as compared to vehicle treated mice (-0.01 ± 0.09h, n=4).
Discussion: Together our results suggest that MLT phase shifts neuronal firing rhythms in the SCN brain slice via direct activation of MT_2 MLT receptors within an SCN oscillator while phase shift of circadian activity rhythms requires activation of MT_1 MLT receptors on an output pathway and/or a circadian oscillator localized outside the SCN. The need for activation of distinct MLT receptors to phase shift overt circadian rhythms or rhythms of neuronal firing within the SCN have important implications for the selection of circadian markers when assessing the effect of MLT treatments on humans circadian rhythms and for the design of drugs to treat circadian rhythms disturbances in affective disorders. *Supported by MH 42922 and MH 52685.*

153. Hippocampal Erk Signalling in Stress-Enhanced Aversive Learning

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Sponsor: William McKinney

Background: The formation of memories of aversive events may be significantly enhanced by prior stressful experiences, as revealed by the acquisition of intense fear responses. If persistent or exaggerated, such responses may lead to the generation of anxiety. In the present study we investigated the role of mitogen activated extracellular signal-regulated kinases (Mek1/2) and their downstream targets extracellularly regulated kinases 1 and 2 (Erk-1/2) and p90-ribosomal-s-kinase-1 (p90Rsk-1) in the effects of stress on aversive learning.
Methods: Experiments were performed in 9 week-old, individually housed male mice. An acute 1 h-immobilization was employed as a stressful stimulus and one-trial context-dependent fear conditioning was used as a model for aversive learning. For pharmacological experiments, mice were implanted with double guided cannula at stereotaxic points corresponding to the dorsal hippocampus. The phosphorylation and distribution of Mek-1/2, Erk-1/2 and p90Rsk-1 were monitored by immunohistochemistry and immunoblot.
Results: Stress triggered a transient double phosphorylaton of Erk-1/2 that was indistinguishable from control levels of these kinases after 3 hr. Nevertheless, the alterations of Mek/Erk distribution and partial phosphorylation persisted during this time period. Training of Balb/c mice 3 h after the end of immobilization enhanced conditioned fear, as indicated by significantly increased freezing behavior of stressed when compared to non-stressed mice. The activation of Erk-1/2 after fear conditioning of stressed mice was significantly facilitated when compared to the non-stressed controls, as revealed by rapid and robust increases of the levels of double phosphorylated Erk-1/2 and their downstream substrate p90-Rsk-1. Intrahippocampal (i.h.) injection of the selective Mek-1/2 inhibitor U0126 prevented stress-enhanced fear conditioning and Mek-1/2-dependent activation of Erk-1/2 and p90Rsk-1.
Discussion: The delayed effects of stress have been so far ascribed predominantly to the alteration of gene expression leading to neuronal remodelling. Persistent alterations of the distribution and partial activation of signalling molecules, such as Mek/Erk, may represent a novel priming mechanism by which stress facilitates the responses of this pathway to subsequent aversive stimuli and thereby enhances the consolidation of conditioned fear.

154. Mood Stabilizers That Downregulate the Brain Arachidonic Acid Cascade Reduce AP-2 or NF-κB Transcription Factors in Rat Frontal Cortex

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Sponsor: Stanley Rapoport

Background: Lithium (Li^+), carbamazepine (CBZ) and valproate (VPA) are mood-stabilizing agents used to treat bipolar disorder.

These drugs have some overlapping biochemical and molecular targets, but whether they have a common mechanism of action is not clear. Therapeutic concentrations of Li^+ and CBZ decrease arachidonic acid turnover brain phospholipids of awake rats, as well as protein and mRNA, and activity levels of cytosolic phospholipase A_2 ($cPLA_2$). VPA also decreases brain arachidonic acid turnover, but does not alter $cPLA_2$ expression; it downregulates COX-2 protein, mRNA and activity.
Methods: We examined the basis for the downregulation or lack thereof of the $cPLA_2$ gene by measuring $cPLA_2$-regulating brain transcription factors (AP-1, AP-2, GRE, NF-κB and PEA3) and in brains of rats administered each of the 3 drugs chronically, to produce therapeutically relevant brain/plasma drug concentrations. Male Fischer-344 rats were fed a LiCl (1.7 g/ kg BW for 2 weeks; 2.55 g/kg BW for 4 weeks)-containing diet for 6 weeks, or were administered CBZ (25 mg/kg) or VPA (200 mg/kg) daily for 30 days. Control rats received a LiCl-free diet or vehicle, as appropriate.
Results: LiCl or CBZ reduced AP-2 brain transcription factor activity and decreased protein levels of AP-2 α and AP-2 β, whereas CBZ decreased AP-2α subunit protein levels. Neither drug changed brain GRE, NF-κB or PEA3 levels. The AP-1 transcription factor also was significantly increased by Li^+ and VPA. VPA did not change AP-2 expression, but decreased brain NF-κB transcription factor activity as well as cytosolic and nuclear p50 levels.
Discussion: The results suggest that Li+ and CBZ reduce brain $cPLA_2$ gene expression by downregulating of its AP-2 transcription factor expression. VPA, which changes neither $cPLA_2$ activity or AP-2 expression, decreases brain NF-κB level, which may explain why VPA downregulates brain COX-2 expression. References: Rao et al. Neuropsychopharmacology, 2005; Rapoport & Bosetti Arch Gen PsychiatrY 59, 592, 2002. Rintala et al. Neuroreport 10, 3887, 1999.

155. Identification and Characterization of D2 Dopamine Receptor Interacting Proteins

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Background: It is evident that dopamine receptors (DARs) do not exist as singular independent units within the synaptic membrane, but rather are part of a large macromolecular complex of interacting proteins. These interacting proteins may be transmembrane or cytosolic, and can play a variety of roles in receptor function, including targeting, trafficking and anchoring, ligand selectivity, association with downstream signaling machinery, specificity of cellular response, and desensitization of receptor response/internalization.
Methods: We have employed a co-immunoprecipitation assay for D2 DARs (from mouse brain and transfected cell lines), coupled with mass spectrometry (MS) sequencing to identify interacting partners. Negative controls were used to limit false positive identification of interacting proteins, and included species and class matched non-immunized antibodies, mock transfected cells, and knockout animals.
Results: Following immunoprecipitation of the D2 DAR from cells or brain tissue, the protein complex was separated using 1D gel electrophoresis and stained with colloidal Coomassie dye to detect proteins. Independent bands were excised, de-stained, trypsinized, and subjected to MS-based peptide sequencing, which yielded detection and subsequent peptide-matching to more than 50 proteins by searching against a non-redundant protein database. In addition to the discovery of novel protein interactors, MS analysis also positively identified the D2 DAR. Several proteins were found in immunoprecipitates from negative controls, and were therefore excluded as non-specific. Novel interacting proteins identified through these experiments include the molecular chaperone protein calnexin and the alpha-1 subunit of sodium-potassium ATPase (NKA alpha-1); co-immunoprecipitation followed by Western analysis has confirmed specific D2 DAR interactions with each of these proteins.
Discussion: Studies are currently in progress to identify interacting proteins in additional neuronal systems and to determine the roles of calnexin and NKA alpha-1 on D2 DAR function and expression. These results confirm the notion that the D2 receptor exists in a "signalplex" consisting of multiple interacting proteins.

156. Protein Interactions of the Vesicular Glutamate Transporter, VGLUT1

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Sponsor: Robert Edwards

Background: Synaptic transmission mediates information processing in the nervous system and involves the regulated exocytotic release of neurotransmitter from synaptic vesicles. Exocytic release of the principal excitatory neurotransmitter glutamate depends upon its transport from the cytoplasm into synaptic vesicles by a family of vesicular glutamate transporter proteins, VGLUTs, located on the vesicle membrane. VGLUT1 and 2, which account for the exocytotic release of glutamate by essentially all well-established excitatory neurons, exhibit a mutually exclusive pattern of expression in adult brain that correlates with the probability of transmitter release and the potential for plasticity. All of the VGLUTs exhibit similar transport activity, but VGLUT1 contains several polyproline protein interaction domains not found in VGLUT2 or 3. The interaction of polyproline motifs with SH3 domain containing proteins contributes to many cellular phenomena, including cytoskeletal organization, the localization of signaling cascades, and the recycling of synaptic vesicles. We have found that VGLUT1 interacts with the SH3 domains of endophilins, proteins previously implicated in endocytosis and synaptic vesicle recycling.
Methods: To identify proteins interacting with VGLUT1, the entire VGLUT1 C-terminus was used as bait in a yeast two-hybrid screen of a rat brain cDNA library. To confirm the interactions observed in yeast biochemically, assess specificity, and to identify the sequences involved, pull-down experiments were performed. To examine the exocytosis and endocytosis of VGLUT1, we used optical methods using fluorescent indicators of synaptic vesicle recycling. Primary hippocampal cultures were transfected with VGLUT1 or a mutant deleting the interaction domain and imaged after field stimulation or potassium application to induce exocytosis of synaptic vesicles. The time course of the fluorescence response of individual boutons during and after stimulation was used to measure the rate of exocytosis and internalization of VGLUT1.
Results: We have found that one of the polyproline motifs in the C-terminus of VGLUT1 interacts specifically with the SH3 domains of endophilins. Endophilins can promote the negative curvature in the membrane required at the neck of budding synaptic vesicles and recruit other proteins involved in membrane recycling. The role of endophilin in endocytosis suggests that the interaction with VGLUT1 promotes internalization of the transporter. While deletion of the interaction domain does not change localization or exocytosis of the transporter, the endophilin interaction does promote efficient internalization of VGLUT1 after exocytosis.
Discussion: These experiments provide some of the first information about the signals that influence synaptic vesicle protein recycling. The presence of the interaction domains in VGLUT1 but not VGLUT2 suggests that the isoforms differ in their membrane trafficking. These motifs may contribute to differences in the rate of vesicle filling, exocytosis and endocytosis, which together determine the ability of the nerve terminal to maintain transmitter release during repetitive firing. Regulation of the rate of vesicle recycling may play a role in the generation of some forms of synaptic plasticity, and so contribute to the changes in synaptic strength that underlie behavioral phenomena and diseases such as learning and memory, drug addiction and schizophrenia.

157. Dexras1 Is a Novel Modulator of Neuronal Adenylyl Cyclases
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Background: Dexras1 is a novel member of the Ras family of monomeric G proteins. Previous studies have revealed that Dexras1 targets both inhibitory G protein alpha subunits and G beta-gamma subunits.
Methods: In the present studies we examined the effects of Dexras1 on the signaling of two neuronal adenylyl cyclases, adenylyl cyclase type 1 (AC1) and adenylyl cyclase type 2 (AC2) in HEK293 cells.
Results: Acute activation of AC1 by the Ca2+ ionophore, A23187 (EC50 ca. 1 uM), was not affected by Dexras1 expression. Additionally, Dexras1 failed to alter beta-adrenergic receptor-stimulated activation of AC1 (Isoproterenol; EC50 ca. 10 nM) or D2L receptor-mediated inhibition of AC1 activity (Quinpirole; IC50 ca. 5 nM). In contrast, Dexras1 inhibited quinpirole-mediated activation of ERK 1/2 approximately 50% and blocked G beta-gamma-dependent heterologous sensitization of AC1 more than 85%. These data suggest that Dexras1 regulation of AC1 activity may occur through the selective inhibition of G beta-gamma-dependent pathways. We also examined the effects of Dexras1 on the activity of AC2. Consistent with the results observed for AC1, Dexras1 did not significantly alter isoproterenol-stimulated AC2 activity, as cyclic AMP accumulation was comparable in the absence and presence of Dexras1. However, Dexras1 completely abolished D2L receptor-mediated potentiation of AC2 activity induced by co-stimulation with isoproterenol and quinpirole. These data are consistent with the ability of Dexras1 to interfere with receptor-mediated activation of G beta-gamma signaling effectors. We also identified a novel role for Dexras1 in modulating AC2 activity by negatively regulating protein kinase C (PKC) signaling. Using the isoform-selective activator of AC2, phorbol 12-myristate 13-acetate (PMA), we demonstrate that Dexras1 abolished PMA-stimulated AC2 activity. The mechanism appeared to be through inhibition of PKC autophosphorylation. Moreover, the role for Dexras1 in regulating PKC was selective for the delta isoform. Additional studies revealed that the effects of Dexras1 required membrane localization and appeared to involve a direct interaction with PKC delta. These data provide evidence that Dexras1 functions to negatively regulate AC2 activity by inhibiting G beta-gamma and PKC delta activity.
Discussion: In summary, we have identified three additional targets of Dexras1. That AC1, AC2 and PKC delta are highly expressed in the central nervous system suggests Dexras1 may serve as an important regulator of neuronal signaling.

158. Long-Term Efficacy of Ziprasidone in Treatment-Refractory Schizophrenia
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Background: Despite the many advances in the treatment of psychosis, as many as 40% of patients with schizophrenia remain treatment-refractory or resistant to treatment with conventional antipsychotic medications (Hellewell, 1998; Bondolfi et al, 1996). Treatment refractory subjects may require longer periods of treatment to adequately assess efficacy; therefore, long-term studies are required. Ziprasidone is an atypical antipsychotic with a unique pharmacological receptor profile: high affinity for D2, 5HT2A, 5HT2C and 5HT7 receptors and partial agonism for the 5HT1A receptor in addition to in vitro affinity for serotonin and norepinephrine transporters.
Methods: Patients were required to have a diagnosis of schizophrenia that was refractory to previous treatment : three or more periods of failure to respond to different classes of antipsychotic agents and no period of good functioning within the preceding five years, a BPRS score ≥45, a score of ≥4 on two PANSS core items and a CGI of ≥5 (moderately ill) at study entry. Subjects were assigned to six weeks of open haloperidol treatment, flexibly dosed up to 30 mg/d (n=415). Subjects who failed to respond (n=306) were randomly assigned to blinded treatment with ziprasidone 80-160 mg/day or chlorpromazine 100 to 1200 mg/day for 12 weeks. Completing patients were then continued on open-label ziprasidone for up to one year. There were 32 patients on ziprasidone who completed the extension study and 30 patients switched from chlorpromazine to ziprasidone.
Results: At the end of the 12-week period, ziprasidone (n=152) demonstrated comparable efficacy to chlorpromazine (n=154) in improving psychotic symptoms and global illness severity. Ziprasidone had significantly greater improvement in negative symptoms ($p \leq .05$) compared to chlorpromazine-treated subjects. Sixty-one percent of the subjects entering the extension phase completed one-year of study. The modal daily ziprasidone dose during the extension phase was 160 mg. Seventy-three percent of the subjects maintained their clinical response over the extension. For subjects initially treated with ziprasidone, significant improvement in PANSS cluster scores (positive, negative, anxiety/depression and excitement) from baseline to last visit (64 weeks; $p \leq 0.0001$) was demonstrated. No mean change in body weight was observed from initial baseline to endpoint (mean 0.23 kg; 4.4 ±SD kg). Mean reduction from baseline in triglycerides was 46 mg/dL ($p \leq 0.001$). Median QTc change was 3.5 msec with no QTc interval ≥500 msec.
Conclusions: In a one-year extension study, subjects with prospectively defined refractory schizophrenia treated with ziprasidone demonstrated significant, continuing, and sustained long-term symptom improvement. Improvement was observed in positive, negative, anxiety, and excitement clusters of symptoms. Ziprasidone had a weight-neutral and favorable effect on metabolic measures throughout the study. Ziprasidone, at 160mg/day, may offer a useful treatment option for subjects who have remained refractory to other treatments. Further study at doses greater than 160 mg/day are warranted in this difficult to treat population.

159. Dopamine and Serotonin Receptor Occupancy of Long Acting Injectable Risperidone in Patients with Schizophrenia
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Background: Long-acting injectable risperidone (Risperdal CONSTA™) is the first long acting formulation of an atypical antipsychotic medication available in the US. It is administered every two weeks as an intramuscular injection with three cycles of injections required to reach steady state. Dynamic F18 PET with fallypride were used to measure D2/D3 receptor occupancy and with altanserin for 5HT2 occupancy of risperidone at steady state. Fallypride has improved sensitivity for measuring D2/D3 binding in limbic and extrastriatal regions that may be differentially affected in schizophrenia and by antipsychotic medication.
Methods: Nineteen patients with schizophrenia (6 women & 13 men - aged 25-55 years) (mean±SD: 40.2±7.3) participated in a randomized blinded clinical trial in which they received 25mg (n=6) or 50mg (n=13) of long-acting injectable risperidone every 2 weeks. PET scans were taken within 3 days of the injection date for determination of peak levels, and within 5 days before the next injection for trough levels. Patients were recruited from three different sites (UC Irvine Medical Center, Synergy, and Health Quest). Nine healthy normal subjects (aged 35.4 ± 8.0) were scanned twice to determine maximum receptor binding. The dynamic PET scanning sequence on the GE2048 PET scanner for 18F-fallypride consisted of 34 frames and 27 frames for 18F-altanserin. The brain regions analized for D2/D3 receptors were putamen, caudate, nucleus accumbens, temporal cortex, and thalamus. The brain regions analized for 5HT2 receptors were senso-

rimotor cortex, anterior cingulate, parietal cortex, occipital cortex, lateral termporal cortex. The reconstructed dynamic PET data sets were aligned and motion corrected using MEDx (a Medical Image Processing and Analysis software), then regions of interest (ROI) were drawn. The time activity curves from the ROI were obtained; the Distribution Volume Ratio (DVR) calculated using the cerebellum as reference. Preliminary analysis on dynamic datasets (Logan et al., 1996) determined the time at which the functional equations became linear for each ROI. DVR of the schizophrenic subjects using the Logan plot were compared to the mean DVRs of the normal controls to calculate occupancy.
Results: D2 Receptor occupancy varied from 57.5% to 83.6%, and were highest in the nucleus accumbens ($p<0.006$, Greenhouse-Geiser). There was no differences between peak and trough D2 occupancy between the 25 mg and 50 mg doses except for the nucleus accumbens where peak occupancy, 83.6%, was higher than trough occupancy, 77.8% ($p\leq.03$). The 5HT2A occupancies varied from 68.2% to 86.3% and were similar in all brain areas. For the 50 mg risperidone group, the parietal and lateral temporal cortices occupancy at peak was higher than trough levels ($p<.04$).
Discussion: These binding data suggest that both the 25 and 50 mg doses of long-acting risperidone achieve a level of receptor occupancy consistent with those previously reported for therapeutic effect. The 50 mg dose results in higher occupancies for D2 and 5HT2A receptors than the 25 mg dose in selected brain areas. The nucleus accumbens had the highest D2 receptor occupancy ($p\leq.006$) of the brain areas assessed and the anterior cingulate had the highest 5HT2A occupancy (ns). The role of receptor occupancy in limbic structures may differ from that in other brain areas and requires further study and confirmation.

160. Adult Outcome in Children with Obsessive-Compulsive Disorder

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Background: Prevalence of OCD in pediatric populations ranges from 1-3%. This prevalence is equivalent to that observed in the adult population. Since the majority of adulthood OCD cases arise after the teenage years, this data suggests that many of the childhood onset cases of OCD may remit by adulthood. Indeed, a recent meta-analysis of previous follow-up studies of childhood OCD suggests that as many as 40-59% of cases will remit by adulthood. However, previous prospective, follow-up studies of long-term outcome of pediatric obsessive-compulsive disorder have had follow-up intervals that ranged from only 1-5 years. We are conducting the current study to determine adult-outcome, a decade or longer after initial evaluation, in children treated for OCD at the Yale Child Study Center.
Methods: We intervierwed more than 50 adults, who had been previously evaluated and treated for OCD as children at the Yale Child Study Center, approximately 10 years after initial evaluation. Initial evaluation consisted of detailed clinical interviews including measures of OCD, tic and ADHD severity, focused neuropsychological evaluations and 1.5-T structural MRI scans. Follow-up clinical interviews included measures of OCD severity as well as comorbid tics, ADHD, depression, anxiety and global psychosocial functioning.
Results: The initial results from this study will be present at the ACNP poster session. Our specific goal is to determine clinical predictors in childhood of increased severity and persistence of OCD symptoms into adulthood. We specifically hypothesize that earlier age of onset of OCD, increased severity of childhood OCD symptoms, presence of a comorbid tic disorder, increased childhood IQ and a family history of psychiatric illness will be associated with increased severity of OCD symptoms in adulthood.
Discussion: Determining clinical predictors of adult-outcome in children with OCD may be helpful in confering prognostic information to clinicians and families caring for children with OCD. It may help better answer many parents most pressing question when they come for care, What will happen to my child? We look forward to presenting the initial results of this study at the ACNP meeting.

161. Regulation of Apolipoprotein L2 in Human Drug Abuse

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Background: Human drug abusers experiencing craving, intoxication or withdrawal demonstrate a dynamic recruitment of select neuroanatomical loci in the frontal cortex. Examining transcriptional patterns in this area may thus help provide clues to which genes, gene families and cellular processes are involved in drug abuse.
Methods: Postmortem human frontal cortex (BA10) from forty-two drug abuse cases and thirty matched controls were assayed using ^{33}P-labeled cDNA and the Mammalian Gene Collection (MGC) cDNA Array. A broad panel of toxicological screens and case histories were obtained to characterize individual cases. Each drug abuse case was compared to the four demographically best-matched controls. A separate dissection from the same drug abuse and control cohort was used for semi-quantitative, real-time PCR (QPCR).
Results: The APOL2 transcript was identified as being consistently altered in drug abuse cases. When less stringent criteria were employed, the APOL1 and APOL4 transcripts were also identified as being changed. By hierarchical clustering of transcriptional profiles, three main groups of drug abuse cases were identified. The APOL2 transcript was up-regulated in the first two groups (N=34), and down-regulated in the third group (N=8). Increased APOL2 expression was validated by QPCR for a subgroup of crack-cocaine abusing subjects by history. A small subgroup of cases characterized by a positive THC hair or tissue toxicology showed down-regulation of APOL2 expression.
Discussion: The APOL family of high-density lipoproteins have a central function in cholesterol transport, and altered expression of APOL family members has previously been identified in the neuropsychiatric disorder schizophrenia. We currently demonstrate up-regulation of APOL2 mRNA in the frontal cortex in the majority of drug abuse cases, with a small subgroup of cases (characterized by a positive THC hair or tissue toxicology) displaying down-regulation. This suggests that lipid metabolism and signaling may be perturbed in the frontal cortex in drug abuse.