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Panel Session
Co-Morbid Pain and Addiction: Novel Treatments

Clinically Significant Advances in Understanding Opiate Receptors

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Opiates are widely used in the treatment of pain. Clinicians have long recognized the need to individualize therapy based upon the widely varying responses of individual patients. However, the reasons for this have remained obscure. Most of the opiates used clinically are selective for mu, or morphine-preferring, receptors, raising the question why the disparities in response among patients can be so great. Another question among clinicians involves the use of Opioid Rotation, in which patients highly tolerant to one opioid can be switched to another with a marked increase in analgesic effectiveness. The utility of this approach appears to rest upon the development of incomplete cross tolerance among opioids, in which the relative potency of one drug to another changes in tolerant compared to naïve patients. These clinical observations can be replicated in a variety of animal models. Perhaps the most dramatic example is the CXBK mouse, which is insensitive to morphine, but retains full sensitivity to other mu opioids, including heroin, methadone and fentanyl. One possible explanation for these observations involves multiple subtypes of mu receptors, a concept first proposed over 25 years ago. Initial work utilized traditional pharmacological approaches, including detailed binding studies and novel antagonists capable of selectively blocking some mu actions and not others. However, the cloning of the mu opioid receptor MOR-1 has opened new insights into the actions of these drugs. MOR-1 encodes a traditional 7 transmembrane G-protein coupled receptor. To date, over 20 splice variants of MOR-1 have been reported in mice and almost a dozen in humans. All the full length variants display the anticipated selectivity and affinity for mu opioids, which is not surprising since the seven transmembrane domains are all identical within each species. Their differences are restricted to the tip of the intracellular C-terminus. However, these variants differ in their localizations within the CNS and within the cell and can be distinguished from each other functionally as well. These studies illustrate the complexity of the mu opioid system. It will be interesting to see if these variants may lead to differences in opioid actions other than analgesia, including reward and addictive potential. Although they cannot yet be used to predict the optimal agent for a specific patient, they provide insights into why we must continue to individualize therapy.

Endogenous Opioids and Dopamine in the Response to Pain in Humans

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This presentation will focus on the function of two neurotransmitter systems, the endogenous opioid and dopaminergic (DA), implicated in response to pain in human subjects, as well as in the effects of opiates and other drugs of abuse. Mu-opioid and DA D2 receptors were quantified at baseline and during pain in healthy subjects using external imagining techniques (positron emission tomography and selective DA D2 and μ-opioid receptor radiotraces). New data will be presented on the involvement of these neurotransmitter systems in responses to sustained pain, a physical and emotional stressor, and their dysregulation in chronic pain conditions. In addition, we will discuss the psychophysical implications of inter-individual differences in DA D2 and μ-opioid receptor availability and in the capacity to activate these neurotransmitter systems. These will be discussed in the context of the contribution of sex, gonadal steroids, common genetic polymorphisms and cognitive factors to these individual variations. Regarding the latter, we will discuss recent and new data on the effects of expectation of relief on the function of these neurotransmitter systems and associated neuronal circuits and their contribution to individual differences in placebo responding. Examination of these cognitively-modulated, resiliency mechanisms further provides a novel avenue for the development of treatment strategies and the understanding of treatment non response in various neuropsychiatric conditions.

Functional Neuro-Imaging of Chronic Pain and Opiate Response

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Opioids are one major class of drugs used in the treatment of chronic pain. Currently there is great concern about the use of opioids in the treatment of non-malignant chronic pain at a patient level (opioid phobia), clinical/provider level (physicians being reluctant to prescribe because of Drug Enforcement Agency (DEA)), related rules or scrutiny and a societal level (opioid addiction is costly). Over the past 5 years opioid prescription drug abuse has evolved into a national epidemic. Chronic pain treatment with opioids is a clinically unusual pharmacotherapy in the sense that we administer a drug class that is known to have addictive properties, and furthermore, do this on a long-term basis. This situation raises a number of important questions including: (1) Are chronic pain patients more prone to becoming addicted to drugs of abuse? Chronic pain is known to produce a number of changes in central nervous systems (e.g., chronic stress) alterations in neural circuitry such as loss of neurons in frontal lobe and thalamus that could affect susceptibility to addiction. In addition, these patients are at greater risk simply through sustained access to prescription drugs and because the opioids commonly prescribed for chronic pain such as oxycontin and hydrocodone seem to have a higher level of abuse, or are considered more "attractive". (2) Why don't all chronic pain patients receiving chronic opioids become addicted? Is there something special about the changes in neural circuitry in chronic pain that protects many patients against addiction despite prolonged use? Links between opioid addiction and chronic pain may be considered at a number of levels including: (1) each on its own produces alteration in brain function; (2) each condition (chronic pain or addiction) may independently result in alteration in reward function; and (3) chronic opioid use, even without pain can produce significant changes in the response to acute pain and clinical pain as observed in methadone maintenance patients. The segregation in the approach to chronic opioid use or abuse is very different amongst the two main clinical groups who see these patients: pain and addiction specialists. As a result of differences in clinical practice approaches by pain clinicians and addiction specialists, numerous patients fall between the cracks. In this presentation we will present new data on the use of functional magnetic resonance imaging (fMRI) in two domains: (a) the role of reward circuitry in acute and chronic pain and (b) the ability to evaluate the effects of opioids on CNS function in human and animal models. The insights gained from these approaches indicate that fMRI may provide an objective measure of alterations in neural circuits that may differentiate "addicted" brain circuits from "non-addicted" brain circuits in patients

with chronic pain and provide a method for validation of tools used to monitor patients in the clinic.

Answering the Key Questions About Co-Morbid Pain and Addiction: Intersection of Clinical Research, Epidemiology, and Policy
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This enormous gap between vigorous laboratory research and scant clinical research on pain and opioid abuse is beginning to close. There has been a rapid acceleration of clinical research in the areas of epidemiology, patient assessment, pharmaceutics, and clinical interventions. Several survey studies on patients in opioid abuse treatment have found the prevalence of co-morbid chronic pain ranging from 25-62%. In pain treatment settings, prevalence of urine toxicology screens suggestive of co-morbid substance abuse has been found to range from 20-40%. An important point beginning to be illuminated by these recent studies is the question of whether an individual without previous addiction can become addicted after exposure to prescription opioids. While there is little direct information, two studies have reported the proportion of patients being treated for opioid addiction who did not have substance abuse disorders prior to exposure to prescription opioids ranges from 10-25%. A number of new approaches with regards to the development and validation of scales to measure the extent of prescription opioid abuse among pain patients are emerging. The SOAPP is a newly validated screening tool to predict prescription opioid abuse in medical practice. The recently developed Version 2 has been found to have a sensitivity of 0.81 and specificity of 0.68. A second instrument called the COMM has been developed as an assessment of whether a pain patient is currently abusing his/her prescription opioids. The recently reported validation study found that the scale had a Coefficient α of .86, test-retest reliability ICC of .86, sensitivity of .77, and specificity of .66 (at a cut-off score of 8). The traditional view that patients with pain do not develop euphoria from opioids has only recently been examined. A small recently conducted pilot study compared patients who received opioids for pain who went on to develop prescription opioid addiction to patients who received opioids for pain who did not develop addiction. The study found that in contrast to the traditional view, addicted patients reported that at the time they first received opioids they experienced significant levels of euphoria (based on ARCI inventory) compared to non-addicted patients.

Panel Session
Determinants of Vulnerability to Nicotine Addiction in Schizophrenia

The Epidemiology of Tobacco Use in Schizophrenia
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A meta-analysis of studies demonstrated that schizophrenia is consistently and strongly associated with tobacco smoking behaviors all over the world. Forty-two samples of schizophrenia patients provided a current smoking prevalence of 62% (4686/7593) and a world average odds ratio (OR) of 5.3 for current smoking when compared with the general population. Out of the 42 ORs, 37 were significantly higher than 1.0. The increased current smoking in schizophrenia reflects increased smoking initiation and decreased smoking cessation. Survival analyses of U.S. and Spanish samples suggested that a person with schizophrenia or vulnerable to schizophrenia who is older than 20 years has a higher daily smoking initiation risk than controls. Moreover, the association between smoking and schizophrenia may not be due to prodromal changes. In two survival analyses, after age 20, smoking initiation rates were higher in schizophrenia patients who started daily smoking at least five years before illness onset than in controls. Thus, schizophrenia vulnerability may be associated with increased vulnerability to starting smoking. People from the general population are not good controls in schizophrenia studies unless a careful control of educational levels and gender is performed. After gender stratification, patients with other severe mental illnesses may be a better control group since they are similar to schizophrenia patients in socioeconomic and educational backgrounds and share other confounding factors such as increased alcohol and illegal drug use and treatment settings. When combining 18 studies (from 9 nations), the significant world average OR for current smoking in schizophrenia versus other mental illnesses was 1.9. Three studies used logistic regression to control for confounding factors, providing significant adjusted ORs ranging between 2 and 3. Limited tobacco access can eliminate the strong association between schizophrenia and current smoking in some nations. Of the 5 nonsignificant ORs from 42 OR, three came from Colombian studies comparing current smoking prevalences in schizophrenia versus those in the general population (18%). However, the 3 ORs were not adjusted for confounders. A matched study included 73 schizophrenia patients, 111 patients with mood disorders and 2 controls from the general population. The previously observed lack of association between schizophrenia and current smoking was due to lack of control of important confounding variables because of the absence of a control group. The careful matching that controlled for confounders, suggested that the association between schizophrenia and smoking behaviors can stand in populations with low monetary income and low smoking rates. The matched study also suggested that the association between severe mood disorders (bipolar and major depression) and smoking is not as strong as that observed between schizophrenia and smoking, and may not be observable in countries where people have limited economic resources.

Vulnerability to Nicotine Addiction in Schizophrenia: Self Medication?
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Most schizophrenics are heavy users of tobacco products. More than 80% of patients smoke compared to approximately 25% of the general population. It has been proposed that smoking in schizophrenics may represent a form of self-medication. Schizophrenics use high-tar cigarettes and extract more nicotine by inhaling deeper and smoking the cigarette more completely. Nicotine, administered as either gum or in cigarettes, has been found to normalize a common auditory sensory processing deficit, the P50 deficit and also to improve eye-tracking abnormalities. Recent data suggests that cognitive deficits may also be improved by smoking in patients. Schizophrenics express lower levels of nicotinic receptors in both postmortem brain and in peripheral blood leukocytes. These receptors flux calcium, resulting in downstream effects on gene expression. Utilizing microarray-based analysis, we have evaluated gene expression in postmortem hippocampus from control and schizophrenic smokers and non-smokers. Expression of more than 250 genes was changed in brain of smokers, including genes expressed in excitatory NMDA pathway. In schizophrenic smokers, however, regulation of gene expression was not the same as in controls. We found that smoking in these patients, again including genes in the NMDA postsynaptic density, differentially regulated 77 genes. The pattern of regulation was generally the same. Levels of mRNA for specific genes, including NCAM, calcineurin A-gamma, and the alpha 7 nicotinic acetylcholine receptor subunit were aberrant in schizophrenic non-smokers and were brought to control levels, or normalized, in schizophrenic smokers. These findings are consistent with a hypothesis of self-medication, but normalization of gene expression by smoking in schizophrenics may make them particularly vulnerable to nicotine addiction.

[I-123] 5-IA-85380 SPECT Imaging of Beta2 Nicotinic Acetylcholine Receptors in Recently Abstinent Control & Schizophrenic Smokers

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Background: Schizophrenic patients and individuals who go on to develop schizophrenia have high rates of comorbid tobacco smoking, smoke more cigarettes and extract more nicotine from cigarettes than other smokers. Chronic nicotine exposure upregulates nicotinic agonist binding to brain nicotinic acetylcholine receptors (nAChR). Postmortem studies have showed higher nicotinic agonist binding in the frontal cortex and hippocampus in control smokers compared to nonsmokers, but not in schizophrenic smokers suggesting a dysfunction in nAChR regulation in schizophrenia. The relevance of this upregulation and the failure of it in schizophrenia in relation to clinical symptoms and cognitive deficits is not yet known.
Objective: To determine the effects of tobacco smoking on beta2-nicotinic acetylcholine receptor (Beta2-nAChR) availability in control and schizophrenic smokers compared to control never smokers using [I-123]5-IA85380 ([I-123]5-IA) SPECT imaging.
Methods: Beta2-nAChR were imaged in control never smokers (n =16), control smokers (n =16) and schizophrenic smokers (n=4) using theBeta2 subunit specific nicotinic agonist radiotracer [I-123]5-IA and SPECT. Schizophrenic and control smokers abstained from smoking for approximately 4-9 days (using progressive reinforcement techniques contingent on abstinence which was biochemically verified using cotinine and carbon monoxide levels) to allow sufficient time for residual nicotine to clear from the brain. Schizophrenic smokers were hospitalized to achieve confirmed abstinence. Subjects were studied using SPECT and 5-IA followed by MRI for coregistration of SPECT data. In schizophrenic smokers, cognitive testing (attention, working memory, selective attention and verbal learning and recall) was assessed while smoking as usual, 24hours after quitting, 5 days abstinence and if subjects resume smoking with the goal of correlating cognitive data with regional brain [I-123]5-IA uptake.
Preliminary Results: [I-123]5-IA uptake was significantly higher in cortical brain areas (26-36%), striata (27%) and cerebella (25%) of recently abstinent (4-9 days) control smokers compared to control never smokers. Consistent with post mortem data, healthy smokers have 30% higher beta2-nAChR availability compared to never smokers. Further, consistent with post-mortem studies pilot data (n=4) show that relative to control smokers, [I-123]5-IA uptake was significantly lower in the frontal and occipital cortices ($p < 0.05$), and trended towards towards lower [I-123]5-IA uptake in the parietal, anterior cingulate and temporal cortices in schizophrenic smokers. Interestingly, there was no difference in [I-123]5-IA uptake in the caudate, putamen and cerebellum between the schizophrenic and control smokers.
Conclusions: These preliminary findings may reflect a failure to upregulate B2-nAChR in a region specific manner in schizophrenic smokers, and may contribute to nicotine addiction vulnerability in schizophrenia. Supported in part by R01 DA015577; TTURC P50AA15632, MIRECC and VACHS Schizophrenia Center.

Effects of Cigarette Smoking on Neurocognitive Deficits in Patients with Schizophrenia

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There is increasing evidence suggesting that the high prevalence of cigarette smoking, heavy nicotine dependence, and frequent cessation failure in patients with schizophrenia may relate to trait features of schizophrenic illness, such as neurocognitive dysfunction associated with this disorder. We have conducted a series of human laboratory studies which have suggested that: 1) smoking abstinence is associated with selective impairment of certain types of cognitive performance including visuospatial working memory, sustained attention, and sensorimotor gating (prepulse inhibition) in patients with schizophrenia who smoke as compared to smoking controls; 2) that these effects of cigarette smoking are dependent on neuronal nicotinic receptor (NNR) stimulation, as such effects are blocked by pretreatment with the NNR antagonist mecamylamine. Furthermore, the presence and severity of prefrontal executive cognitive dysfunction before a quit attempt (e.g. deficits in performance of WCST, visuospatial working memory, Trails B or Digit Span-Backwards) has been associated with a decreased likelihood of smoking cessation in two independent smoking cessation trials from our group at Yale University, a finding not observed in non-psychiatric control smokers. This suggests that relatively intact prefrontal executive function is required to learn the skills to quit smoking in these patients, and that such deficits constitute a vulnerability factor to smoking cessation failure. Collectively, our findings suggest that the development of therapeutic agents based on NNR systems (but without the harmful effects of tobacco) may lead to better pharmacological treatments for cognitive dysfunction in schizophrenia, and for the treatment of comorbid nicotine dependence in this disorder. Supported in part by grants R01-DA-14039, R01-DA-13672 and K02-DA-16611 from NIDA/NIH (to TPG) and NARSAD Young Investigator (to KAS) and Independent Investigator (to TPG) Awards.

Panel Session
New and Differing Roles for Brain-Derived Neurotrophic Factor in Cocaine Addiction

The Role of the Brain-Derived Neurotrophic Factor/Dopamine D3 Receptor Pathway in the Responses to Drugs and Drug-Associated Stimuli

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In addition to its role as a neurotrophin, brain-derived neurotrophic factor (BDNF) appears to function as a neurotransmitter, as suggested by the observations that it is anterogradely transported and released in an activity-dependent manner by neurons and produces rapid synaptic responses. BDNF is largely expressed in the dopamine mesolimbic system, which is an important anatomical substrate for cocaine reinforcement and cocaine-seeking behavior. BDNF supply is provided by both dopamine neurons originating from the ventral tegmental area (VTA) and glutamatergic afferents from the prefrontal cortex (PFC) and amygdala to the nucleus accumbens (Nac). Time-dependent alterations of mesolimbic BDNF after cocaine withdrawal accompany "incubation" of craving and application of exogenous BDNF into the Nac or VTA increases response to cocaine-associated stimuli and induces long-lasting enhancement of cocaine seeking; in contrast BDNF infusions in the PFC attenuates reinstatement of cocaine seeking (see other speakers in this panel). The dopamine D3 receptor (D3R) present in the nucleus accumbens is an important mediator of the responses to drug cues. Thus, several D3R-selective partial agonists and antagonists inhibit operant and non operant responses to exposure to stimuli associated with various abused drugs, including psychostimulants, opioids, nicotine and alcohol, but not with natural reinforcers such as food. These D3R ligands have no intrinsic reinforcing properties and do not interfere with the reinforcing properties of drugs. D3R expression is controlled by BDNF originating from either VTA dopamine neurons or PFC glutamate neurons. Exposure to drugs elicit a transient increase in BDNF expression in the PFC, but a long-lasting increase in D3R expression in the Nac, whereas exposure to drug cues elicits persistent increase in expression of BDNF in the VTA and D3R in the Nac. However, chronic systemic infusion of the psychotomimetic drug MK-801 also

elicits activation of the PFC to Nac BDNF/D3R pathway, which is accompanied by PFC dysfunction and psychotic-like behavioral disturbances, which are corrected by D3R-selective ligands or antipsychotic drugs. These results suggest that activation of the BDNF/D3R pathways may facilitate drug cue-induced drug seeking or craving in addiction, but that the anatomical location of the primary changes, VTA or PFC, may modulate the behavioral outcomes.

A Single BDNF Infusion into the Medial Prefrontal Cortex Suppresses Contextual-, Cue-, and Cocaine-Induced Reinstatement of Cocaine-Seeking Behavior

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Chronic exposure to cocaine induces long-lasting neuroadaptations within the mesocorticolimbic and mesocorticostriatal pathways that are thought to underlie the emergence and expression of addictive behaviors. Infusion of brain derived neurotrophic factor (BDNF) within the nucleus accumbens (NAc) or the ventral tegmental area (VTA) has been demonstrated to augment cocaine reinforcement and the reinstatement of cocaine-seeking behavior. However, the dorsal striatum (caudate-putamen) and ventral striatum (NAc) receive most of their BDNF by anterograde transport from corticostriatal, not mesostriatal, pathways. Yet little is known about the role of BDNF in the projections from the pre-frontal cortex (PFC) to the dorsal and ventral striatum that are critical to relapse behaviors. Therefore, in the present study, rats self-administered cocaine (0.2 mg/inf) on an FR1 schedule of reinforcement, with contingent presentation of a light and tone, during 2 hour sessions per day for 10 days. Immediately after the 10th self-administration session, rats received bilateral intra-PFC or intra-VTA infusions of BDNF (0.75μg/0.5μl/side) or vehicle (PBS, 0.5μl). Following 7 days of abstinence in the home cage, rats were re-exposed to the self-administration environment in the response-contingent presence of the conditioned light and tone stimuli during a 2-hour session. Rats that had received intra-PFC infusions of BDNF exhibited significantly less active lever responding during this test relative to vehicle-treated rats. Similarly, rats that received intra-PFC infusions of BDNF exhibited less lever responding following extinction and a conditioned cue (tone and light) test or a cocaine challenge (10 mg/kg, i.p.). This evidence sharply contrasts with animals that received intra-VTA infusions of BDNF in which reinstatement behaviors were augmented or not changed. These data implicate BDNF in the PFC as an important suppressor of relapse to cocaine seeking. Supported by P50 DA015369.

Dynamic Regulation of Nucleus Accumbens Brain-Derived Neurotrophic Factor Produces Enduring Addiction-Related Changes in Cocaine Self-Administration and Relapse

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Chronic psychostimulant use produces both transient and enduring neuroadaptations in the mesolimbic dopamine pathway that may differentially contribute to addiction-related escalation in cocaine self-administration (SA), and enhance the propensity for relapse in prolonged abstinence. Brain-derived neurotrophic factor (BDNF), a downstream target of CREB-regulated gene transcription, could induce long-term neuroplasticity that underlies persistent behavioral changes in cocaine addiction. While normal levels of BDNF expression in striatal neurons are very low, we found that daily intravenous cocaine SA (4 hrs/day) induced a 2-3 fold increase in BDNF mRNA levels in both core and shell subregions of the nucleus accumbens (NAc), while proBDNF (precursor) protein levels increased by 26%, but only in the shell subregion. Cocaine-induced elevations in BDNF protein were paralleled by TrkB-mediated PLCγ phosphorylation, an effect blocked by intra-NAc anti-BDNF infusions, suggesting that newly synthesized BDNF is released by cocaine SA. Both BDNF levels and PLCγ phosphorylation returned to basal levels 24 hrs after cocaine SA. Thus, NAc BDNF activity rises and falls following each cocaine SA binge. To study the role of this dynamic BDNF regulation in addictive behavior, we tested the effects of repeated daily intra-NAc BDNF and ANTI-BDNF infusions on subsequent cocaine-taking and -seeking behaviors in self-administering rats. Following acquisition and stabilization of cocaine SA, animals received five daily intra-NAc shell microinfusions of BDNF (2.5μg/0.5μl/side), ANTI-BDNF (5μg/0.5μl/side) or PBS/IGG, immediately after each 4-hr SA session. The following week, animals were tested in either fixed or progressive ratio cocaine SA dose-response procedures. In fixed ratio testing, BDNF-treated animals exhibited a vertical and rightward shift in the inverted U-shaped dose-response curve, including an escalation in cocaine intake and higher peak SA rates, similar to phenotypic changes reported in other models of cocaine addiction. Conversely, blockade of cocaine-induced elevations in endogenous BDNF with ANTI-BDNF infusions led to a downward shift in the inverted U-shaped dose-response curve. In a separate group of animals, repeated intra-NAc BDNF infusions subsequently increased in the amount of effort animals exert to obtain reinforcement at high cocaine doses on a progressive ratio reinforcement schedule when tested several days following the BDNF infusions. In addition, prior intra-NAc shell BDNF treatment during SA training subsequently facilitated reinstatement of cocaine seeking induced by cocaine priming and footshock stress, while prior anti-BDNF treatments attenuated the propensity for cocaine seeking. Similar BDNF treatment in the caudate-putamen failed to alter these cocaine-taking and -seeking behaviors, and intra-NAc shell infusions of BDNF produced no effect on reinstatement of sucrose-seeking behavior. To determine the role of endogenous BDNF derived from NAc neurons on cocaine reinforcement, we used viral-mediated expression of cre-recombinase in adult floxed BDNF mice to produce a highly localized knockout of BDNF, verified by rtPCR of laser captured cre recombinase-expressing NAc neurons. Localized knockout of BDNF in NAc neurons led to a downward shift in cocaine SA dose-response curves (fixed ratio), suggesting a necessary role for NAc-derived BDNF in cocaine reinforcement. Together, these data suggest that dynamic regulation of BDNF expression in NAc neurons during chronic cocaine SA contributes to increased cocaine intake, enhanced cocaine reinforcement, and an enduring propensity for cocaine seeking behavior in long-term cocaine withdrawal.

Roles of BDNF Within the Mesolimbic Dopamine System in Cocaine Craving and Relapse

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Brain-derived nerve growth factor (BDNF) is critical for neuronal survival and differentiation; it also plays pivotal roles in the maintenance and remodeling of neuronal functioning in the adult brain. BDNF was recently found to enhance responding for cues associated with natural rewards, which suggests that BDNF may be involved in mediating long-term cellular and behavioral adaptations associated with drug addiction. Using a rat relapse model, we found that cocaine seeking induced by exposure to cocaine-associated cues progressively increases after withdrawal. This progressive increase is associated with increases in BDNF levels within the mesolimbic dopamine system. Based on these findings, we further studied whether BDNF infusions into the ventral tegmental area (VTA), the cell body region of mesolimbic dopamine neurons, would potentiate cocaine seeking after withdrawal. Rats were trained to self-administer cocaine for 10 d, and cocaine seeking was measured in extinction tests 3, 10, or 30 d after withdrawal. During testing, rats were exposed to contextual cues that had predicted cocaine availability during training, and lever presses resulted in contingent presentations of a discrete tone-light cue that was previously temporally paired with cocaine infusions.

BDNF (0-0.75 microg/site) or nerve growth factor (NGF; 0-0.75 microg/site) was infused into the VTA 1-2 hr after the last self-administration session. To examine the role of the mitogen-activated protein kinase (MAPK) pathway in BDNF effects, U0126 (1 microg/site), an MEK inhibitor, was used. We found that a single intra-VTA infusion of BDNF, but not NGF, induced long-lasting enhancement of cocaine seeking for up to 30 d, an effect reversed by U0126. In contrast, neither BDNF infusions into the substantia nigra, nor acute intra-VTA BDNF infusions 2 hr before testing on day 3 of withdrawal, were effective. These data suggest that BDNF-mediated neuroadaptations in mesolimbic areas are involved in the persistent cocaine seeking induced by exposure to drug cues after withdrawal.

Panel Session
New Medication Development for Schizophrenia and Mood Disorders: Academia, Industry and FDA Perspectives

Therapeutic Discovery for Mood and Anxiety Disorders: Barriers and Opportunities

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Mood and Anxiety Disorders are among the most prevalent and serious of all medical disorders. Most of the availible treatments were discovered by serendipity and work via similiar mechanisms of action. Moreover, these treaments have significant limitations in efficacy and tolerability.This presentation will briefly review the history of therapeutic discovery for mood and anxiety disorders. More attention will directed toward developing a strategic plan for accelerating the pace of therapeutic discovery via enhancing research directed toward molecular target identification, molecular drug libraries, more precise phenotypic definition of disorders utilizing advances in human genetics and brain imaging, and novel clinical trial designs.

Molecular Targets for Affective Disorders and Anxiety

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The first generation drugs to treat affective disorders (e.g. MAO inhibitors and tricyclics for depression, lithium for bipolar illness) and anxiety (benzodiazepines and buspirone) were discovered decades ago and were generally empirically derived by keen clinical observations. Subsequent drugs such as selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs) which are useful for anxiety as well as depression were iterative advances based on these early agents. Newer approaches for these conditions include many targets and mechanisms which came from our understanding of the genome and for the most part have not yet been validated clinically. Agents for these targets were rationally derived based on hypothetical understanding of disease pathology coupled to cloning of receptors that allowed for the discovery of novel pharmacological tools that were active in animal models. These animal models for the most part have been validated only with older clinically effective agents, suggesting that their usefulness to find novel agents across the many forms of these illnesses may also be limited. Several of these agents (e.g. glutamate modulators, neuropeptide receptor antagonists) are now being investigated in the clinic. More recently, drugs that target the hypothesized underlying pathophysiology in these diseases, such as impaired or pathologically altered brain plasticity have been envisioned. This presentation will overview and discuss novel molecular targets for depression, anxiety and bipolar illness, including their current status, perceived limitations and challenges ahead, and how they might compare to existing clinically effective agents.

Drug Discovery for Schizophrenia: Barriers and Opportunities

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Background: Drug discovery for schizophrenia has resulted in minimal progress since the 1952 introduction of chlorpromazine. The modest clozapine superiority in treatment resistant patients is the only documented superiority claim, and the partial agonist effect of aripiprazole is the only variation on the D2 antagonism mechanism of action theme. Core pathologies such as primary negative symptoms and impaired cognition have been neglected, and no drug has documented efficacy in these domains. The advantages sometimes observed in comparison to substantial doses of haloperidol tend to vanish with low dose haloperidol comparison, and the FDA has not approved any drug for a cognition or primary negative symptom indication. The purpose of this report is to determine the reasons for lack of progress, and to identify best opportunities for drug discovery for schizophrenia.
Method: National concern with low innovation in drug discovery has prompted the Institute of Medicine to establish a Forum to address barriers and opportunities. Background reports have been commissioned to identify the issues as they relate to schizophrenia/psychosis, depression/anxiety, and molecular targets. The report on schizophrenia is based on a review of paradigms used in drug discovery, ascertainment of key factors which impede innovation, a review of clinical effects of drugs, and a review of animal and human models which may be informative in early proof of principle/concept studies.
Results: Nine barriers were identified including absence of specific molecular pathology knowledge, the influence of the disease entity paradigm, misinterpretation of antipsychotic drug effects on schizophrenia, the lack of ambitious treatment goals, and the substantial market available to new drugs based on the antipsychotic development platform [the "me-too" problem]. Opportunities include a shift in paradigm to domains of pathology including cognition and avolition, establishing development platforms related to domains, relating animal models to specific domains, the application of genotype/phenotype knowledge in development and testing of compounds, a focus on pathologies responsible for long-term morbidity and poor functional outcomes, and development goals that include cure and prevention.
Discussion: Drug development for schizophrenia based on psychosis as a proxy for schizophrenia pathology has resulted in little progress since the introduction of chlorpromazine. An alternative paradigm calls attention to multiple domains of pathology. These domains, rather than schizophrenia as a class, represent the pathologies for which molecular pathology must be identified. At present, these domains represent the clinical therapeutic targets for which drug discover should be focused. These domains stimulate interest in novel mechanisms and provide a framework for relating molecular neuroscience to animal models, human models and clinical targets. Investment in high-risk drug development is required to break from the D2 tradition. This is essential if progress on functional outcomes, recovery, cure and prevention is to be achieved.

Atypical Antipsychotic Drugs: Perils for Preclinical Drug Discovery Strategies

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Introduction: As part of an Institute of Medicine sponsored study, we evaluated preclinical predictors of success and failure for CNS drug discovery. Not surprisingly, CNS drug discovery has, historically, been designated as an extraordinarily risky endeavor. To gain further insight into the reasons for failure and success in CNS drug discovery, we focused on 3 target areas: schizophrenia, depression and anxiety and examined the success rate for drugs that had entered at least the later stages of preclinical research.
Methods: A total of 140 antipsychotics were initially classified based on their molecular target(s) and then evaluated using three overall

criteria: (1) type of preclinical validation; (2) whether or not preclinical studies predicted clinical efficacy; and (3) whether the compound was of greater efficacy than "conventional treatment".
Results: For the treatment of schizophrenia, we found that preclinical models were highly effective at predicting whether or not a candidate molecule would have 'atypical' properties (i.e., produce fewer EPS than a conventional antipsychotic drug such as haloperidol). On the other hand, the various preclinical models were only fair at predicting overall efficacy and ineffective at predicting efficacy greater than 'conventional treatment'. Thus, many classes of compounds (e.g. sigma1-, D1 and D4-selective compounds) were predicted by multiple preclinical animal models to be effective, but were subsequently found to be ineffective in humans. Finally, it was clear that 'multi-receptor' compounds were uniformly more effective than 'single-target' compounds for treating schizophrenia and related disorders (Roth et al., Nature Rev Drug Discov 2004). Because it is difficult to 'design-in' the preferential ability of a drug to hit multiple identified molecular targets, we and others have suggested that novel chemical scaffolds and novel chemistries should be attempted to generate 'multi-receptorial compounds.'
Conclusions: None of the available animals models accurately predicts the propensity of various antipsychotic drugs to induce weight gain and associated side-effects, although this might be able to be predicted based on a knowledge of in vitro receptor pharmacology. Finally, in terms of the domains of efficacy (e.g., improving cognition, diminishing suicidality, diminishing deficit symptoms), none of the commonly used animal models are highly predictive, although preclinical memory models may be useful for predicting the ability of selected agents to enhance cognition, and receptor-based studies might predict whether or not a compound may diminish suicidality. Thus, for instance, the M1- muscarinic agonist properties of N-desmethylclozapine have been proposed to be predictive of the pro-cognitive actions of clozapine. Additionally, the greater potency of clozapine for 5-HT7 receptors can be used to predict the greater efficacy of clozapine versus olanzapine in preventing suicidal behavior. These findings imply that M1-agonists might be useful as 'add-on medications' for enhancing cognition while 5-HT7 antagonists may have anti-suicide actions. It appears to be impossible to predict using the currently available models whether or not compounds lacking D2-dopamine receptor activity (either as antagonists or partial agonists) will prove effective in treating schizophrenia. Additionally, although enhancing cognition in schizophrenia remains a laudatory goal, there is no guarantee that any of the currently available preclinical models will predict efficacy in human domains of cognition in schizophrenia.

Panel Session
RNA Splicing and Processing in Neuropsychiatric Disease

Alternative Splicing of the NR1 Subunit of the NMDA Receptor in Schizophrenia: Implications for NMDA Receptor Processing, Trafficking and Turnover

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Background: NMDA receptor dysfunction has been postulated in schizophrenia and this hypothesis is variably supported by a number of studies that have found abnormal NMDA receptor subunit and binding site expression in the schizophrenic brain. One limitation of these studies is that they do not account for alterations in receptor assembly, trafficking, or turnover, processes that rely, in part, on alternative splicing of the NR1 subunit, as well as a series of protein-protein interactions between specific NMDA receptor subunits and several recently characterized receptor interacting proteins. Alternative spliced variants of the NR1 subunit confer differential intracellular interactions for receptor processing and trafficking. C1 exon-containing splice variants of the NR1 subunit modulate an interaction between the NMDA receptor and neurofilament-light (NF-L), stabilizing the NMDA receptor in the cell membrane. Exclusion of the C2 exon yields a novel stop codon, resulting in a shorter splice variant expressed at the C-terminus (called C2'). This alternative splicing of the C2 cassette regulates NMDA receptor trafficking and cell surface expression. We hypothesize that NMDA receptor abnormalities in schizophrenia are not simply limited to having "too much" or "too few" NMDA receptors, but rather, are a problem of alterations in NMDA receptor cell biology.
Methods: To test this hypothesis, we measured expression of two alternatively spliced isoforms of the NR1 subunit (NR1C2 and NR1C2') and of the NR2A-D subunits of the NMDA receptor, as well as of the NMDA interacting molecules NF-L, SAP102, PSD-95 and PSD-93 in the PFC in postmortem samples from elderly schizophrenic patients and a comparison group.
Results: We found significantly increased expression of NR1C2' but not of NR1C2, suggesting altered NMDA receptor cell membrane expression in the PFC. We did not detect changes of any of the NR2 subunits studied in either cortical area. One implication of variable splicing of the C-terminus is that specific regions of the C-terminus are involved in interactions with regulatory proteins that modulate NMDA receptor function. Thus, we next studied expression of the NMDA interacting molecules NF-L, SAP102, PSD-95 and PSD-93 in the PFC at both transcriptional and translational levels; increased transcript expression was associated with decreased protein expression, suggesting abnormal translation and/or accelerated protein degradation of these molecules in schizophrenia.
Conclusion: Our findings suggest abnormal processing of the NMDA receptor and its associated PSD molecules possibly involving alternative splicing, trafficking and protein stability in cortical areas in schizophrenia.

Postmortem Brain Studies in Schizophrenics and Controls: Splice Variants and Isoforms

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Background: Over the last several years, allelic variations in a number of genes have been identified that are associated with increased risk for schizophrenia, as well as with cognitive deficits that characterize the syndrome. Our lab and others have examined the expression of a number of these genes in both dorsolateral prefrontal cortex (DLPFC) and hippocampus (HC). In particular, we and others have found decreased expression of BDNF in DLPFC (Weickert CS, et al., 2003 and Hashimoto T, et al., 2005) Alternatively, expression of a specific NRG-1 isoform (TYPE I) appears to be increased in both DLPFC (Hashimoto R et al, 2004) and hippocampus (Law AJ et al., 2006). Moreover, allelic variations associated with increased risk for schizophrenia in the original Icelandic haplotype (Stefansson H et al., 2002) are associated with both type I and type IV isoform mRNA expression in these studies (Law et al., 2006). This presentation will focus on the data from NRG-1 studies as well as DLPFC studies of splice variants of BDNF.
Methods: A large cohort of schizophrenic patients (n = 53) and controls (n = 90) have been used for these postmortem studies. Cases are reasonably well matched for a number of variables including age, gender, race, postmortem interval (PMI), pH and RNA integrity. DLPFC and HC were dissected from frozen brains and DLPFC gray matter was dissected from white matter. qRT-PCR was used to determine expression levels of splice variants of BDNF including exons 1-5, 2-5 and 3-5 in DLPFC gray matter and Types I –IV isoforms of NRG-1 in HC. Several control genes were used for each study. Data was analyzed by ANCOVA (covarying for pH, age, PMI for NRG1 studies and age for BDNF studies) followed by post hoc t-tests. Effects

of allelic variations on expression were done with ANOVA followed by posthoc t-tests.
Results: The major findings in this study are as follows: BDNF: 1.Decreased BDNF 2-5 mRNA expression in DLPFC of schizophrenic patients relative to controls ($p < .03$). 2. In schizophrenic patients treated with antidepressants there is increased expression of the 2-5 splice variant in DLPFC ($p<.04$) and a trend for increased expression of the 1-5 splice variant ($p<.07$). 3. No significant changes in mRNA expression in the 3-5 splice variant. NRG1: I. Increased Type I NRG-1 mRNA expression in HC of schizophrenic patients relative to controls ($p=.004$). 2. Allelic variation in SNP8NRG22132, a SNP in the Icelandic NRG-1 haplotype (Stefansson H et al., 2002) is associated with expression of Type I NRG-I mRNA in HC ($p<.003$) as well as in the DLPFC of the original Hashimoto R et al, 2004 study. The effect of this SNP on expression of TYPE I showed different allelic association in controls and in schizophrenic patients. 3. Moreover, another SNP in the same risk haplotype (SNP8NRG243177) and a 22kb block of this haplotype are associated with mRNA expression for the novel Type IV NRG-1 isoform ($p< .04$). (Law AJ et al., 2006). This SNP also is computationally linked to a transcription factor binding site for two transcription factors that have been implicated in NRG-1 function.
Conclusions: Specific splice variants of BDNF and isoforms of NRG-1have altered expression in brains of schizophrenic patients. The relationship of allelic variants to expression levels of these slice variant/isoforms may be important first steps in guiding further cell biology studies that elucidate the neuropathology of schizophrenia.
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Decreased Expression of RNA Binding Proteins in Schizophrenia
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Background: Independent gene expression studies have documented decreases in RNA binding proteins (RBPs) in SCZ brain (Prabakaran et al. 2004, Katsel et al. 2005, Haroutunian et al. 2005). For example, QKI, encoding an RBP essential for normal myelination was found to be severely decreased in SCZ brain in two recent studies (Aberg et al. 2005, Haroutunian et al. 2005). QKI has 3 major splice forms, QKI-5 which is nuclear and QKI-6 and QKI-7 which are cytoplasmic. The cytoplasmic isoforms bind to the 3'UTR of target transcripts to control their translation and the nuclear isoform is involved in splicing. MBP is a well studied Qk1 target and contains Quaking Star Binding Elements (QSBEs). QSBEs are high affinity binding sites for Qk1 (Ryder et al. 2004 and Galarneau & Richard 2005). Several RBPs including SFRS1, HNRNPA1 and SSA2 are decreased along with Qk1 in BA7 and BA36, but not in the ACC, of SCZ subjects. These RBPs also harbor QSBEs. PLP1 and MAG are abnormally spliced in the Qkviable mutant mouse yet SFRS1 is responsible for splicing of PLP1 (Wang et al. 2005), and MAG also harbors similar splicing recognition sites. We hypothesize that splicing of PLP1 and MAG are aberrantly spliced in BA7 and BA36 but not in the ACC of SCZ subjects.
Methods: A. Bioinformatics approaches were used to identify putative QSBE consensus sites in SFRS1, HNRNPA1 and SSA2. B. Competition fluorescence polarization (FP) binding experiments were performed using chemically synthesized 12mer RNA targets to compare the affinity of each site to that of Qk1 binding to its best site in the 3 UTR of MBP mRNA. C. For the most interesting targets, 30mers were prepared and direct titration binding experiments were performed, both by FP and by gel shift assay. D. Qk1-RNA Co-IPs were performed using a bead binding assay followed by RT-PCR to confirm in vivo targets of QKI. E. Expression of SFRS1, HNRNPA1, SSA2 and isoforms of Qk1, PLP1 and MAG were evaluated in BA7, BA36 and the ACC from SCZ cases versus controls suing QRT-PCR
Results: Two of three possible sites in SFRS1 transcripts bind to Qk1 with affinity as tight or tighter than to the defined MBP sites (Kd, app: 40 ± 3 nM, 14 ± 3 nM, MBP QSBE Kd: 35 ± 4 nM). In addition, the HNRNPA1 site and one of the two candidate SSA2 sites bind with high affinity (Kd, app: 20 ± 5 nM, 38 ± 5 nM). In the case of SFRS1, we have validated the interaction with Qk1 by quantitative EMSA and Co-IPs. The binding constant of Qk1 for one of the SFRS1 QSBEs determined by EMSA is identical to the FP measurement (Kd, app: 17 ± 3 nM). The Co-IPs show that Qk1 binds to MBP and SFRS1 mRNA in mouse prefrontal cortex and hippocampal extracts. We will also show whether splicing of PLP1 and/or MAG is abnormal in BA7 and BA36 but not in the ACC.
Discussion: Decreased expression of Qk1 leads to decreased expression of at least 3 RBP targets with regional specificity in SCZ brain including SFRS1, HNRPA1 and SSA2. We have confirmed that SFRS1 is a true in vivo target of Qk1 and demonstrate the effects of reduced SFRS1 on splicing of its known target, PLP1, and a putative target, MAG, in BA7 and BA36 of SCZ brain.

Multiple Roles of the Mouse RNA-Binding Protein QKI
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Background: For nearly forty years, functional studies of the mouse quaking gene (qkI) focused on its role in the postnatal central nervous system during myelination. The qkI gene encodes for a STAR (signal transduction and activation of RNA)-domain RNA-binding protein, which may function to control of RNA splicing and/or stability. Recently, studies have implicated human QkI in susceptibility to schizophrenia (SCZ). However, the study of mouse ENU-induced alleles reveals that quaking has additional functions. First, the mutants reveal a critical role in blood vessel development, which include retinoic acid-dependent and independent regulation of vascular cell behavior. Secondly, our recent studies reveal a potential role for quaking in neuronal maintenance. Thus, QkI may play a crucial role in many tissues that may impact SCZ, and understanding how alterations in qkI affect such diverse processes is our current goal.
Methods: We use strains of mice, isolated after N-ethyl-N-nitrosourea (ENU) mutagenesis and screening, to study the biological and molecular defects associated with mutation. Effects on mRNAs and proteins are detected by Northern analysis, quantitative real time PCR, and Western blotting. Oligodendrocyte specific proteins are conjugated with fluorescent proteins prior to imaging. Regulatory elements are analyzed after subcloning in a luciferase vector, assayed in an oligodendrocyte cell line, and are confirmed in transgenic mice.
Results: The allelic series of the mouse quaking gene includes the spontaneous quaking viable (qkv) allele, which has a dysmyelination phenotype, four ENU-induced alleles (qkkt1, qkk2, qkkt3/4, and qkl-1) that are homozygous embryonic lethal, and one viable allele, qke5. The original qkv allele is a deletion that eliminates the function of Parkin, the Parkin co-regulated gene, Pacrg, and alters the expression of the flanking qkI gene. The qke5 point mutation provides a useful simple model of dysmyelination. Unlike the qkv/qkv deletion, qke5/qke5 mutants have early onset seizures, severe ataxia and a dramatically reduced life span. Although ultrastructural analysis of qke5/qke5 brains reveals a severe lack of myelin, calbindin staining in the cerebellum of young adult qke5/qke5 mice reveals Purkinje cell axonal swellings indicative of neurodegeneration that are not seen in young adult qkv/qkv mice. Such a finding implies a new role for quaking in axonal maintenance. Further, analysis of myelin components reveals abnormalities in a variety of proteins, including MBP, CNP, and PLP, which are also altered in SCZ brains. The mutant allelic series also indicates that regulation of expression of the quaking gene plays a critical role in its function in different tissues. We have recently found two regulatory elements upstream of quaking

that control its oligodendrocyte-specific expression. The target consensus binding sequence for the QKI protein has recently been identified and we have found this sequence in the 3'-untranslated region (UTR) of the qkI gene. This, along with our finding that when the nuclear isoform is not produced the two cytoplasmic isoforms are affected, suggests that the QKI protein may be regulating splicing of its own mRNA through the use of the consensus binding site.
Conclusions: It is not currently straightforward to model schizophrenia in the mouse, since the physiologic and behavioral manifestations of the disease in a mouse are not known. However, we have noted that two of the alleles, qkkt1 and qkk2, have noteworthy semidominant effects, which include seizure susceptibility. Certainly, understanding the molecular mechanisms of the role of quaking in diverse processes such as myelination, neuronal maintenance or vascular remodeling will aid in our understanding of its role in schizophrenia.

Panel Session
The Fragility of Phases of Memory: Reactivation and Reconsolidation

Understanding How Strength of Trauma Influences That Memory's Ability to Undergo Reconsolidation

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Although memory reconsolidation is a fundamental process, reports of boundary conditions such as strength of training imply that reconsolidation is not ubiquitous. These boundary conditions, however, remain poorly defined across levels of analyses. If we are going to target reconsolidation of traumas as a therapeutic approach, it is very critical to understand how a strong emotional memory interacts with reconsolidation in animal models. Attempting to ameliorate this situation, we characterized reconsolidation of strong auditory fear memories across all three levels of analysis. At the behavioral level we demonstrated initially does not undergo reconsolidation during the first week post-training, but does one month after training. At the systems level we showed that the hippocampus imposes the boundary condition on the amygdala. At the molecular level we demonstrated that the degree of expression of NR2B-containing NMDA receptors in the amygdala modulates reconsolidation of overtrained fear memories, as these receptors, which we previously have identified as being essential for the transformation of a consolidated memory back to a labile state. Furthermore, animals with pre-training hippocampus lesions, that did not exhibit the overtraining boundary conditions two days after training, had normal level of expression of NR2B subunits at that time-point. These findings make three conceptual advances in our understanding of reconsolidation: first, boundary conditions can be transient, second, boundary conditions can be imposed by other brain systems, and third, a mechanism mediating the manifestation of boundary conditions is down-regulation of the receptors that are critical for inducing reconsolidation.

Destabilization of Pathogenic Memories in Animal Models of Trauma and Addiction

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Our studies focus on the characterization of the anatomical, molecular, pharmacological and temporal requirements underlying the fragile phase evoked by the reactivation of an established memory. Specifically, we have investigated two models of pathogenic memories in rats: one reproduces traumatic memories and the other represents memories induced by drugs of abuse. In the first model, we found that the amygdala together with the stress-dependent modulation pathway play a critical role in maintaining memory stability following reactivation. We also found that the disruption of reactivated traumatic memories is more efficient when the memory is recent. In the addiction model, we identified the hippocampus, amygdala and nucleus accumbens, but not the ventral tegmental area, as brain regions where the inhibition of new protein synthesis persistently disrupts the retention of established memories following their reactivation. We are currently exploring whether the disruption of memories induced by drugs of abuse affects other addiction responses. We will discuss results obtained in both models that may help design strategies to develop novel pharmacological and behavioral therapies to treat post-traumatic stress disorders and addiction.

Reconsolidation in Humans?

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Developing techniques to alter previously learned emotional responses and memories by disrupting reconsolidation has tremendous clinical potential. However, there is not yet any clear evidence from controlled laboratory studies in humans for disruption of reconsolidation for emotional memories. I will present our initial attempts to find this evidence. Although these initial studies provide some support for the disruption of reconsolidation in humans, the findings are complicated by a number of factors, including the multiple forms representation that characterize human emotional learning. These results suggest that applying the principles of reconsolidation to the treatment of psychopathology will require an appreciation of the subtleties of human emotional memory.

A Novel Treatment for Post-Traumatic Stress Disorder by Reconsolidation Blockade with Propranolol

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Background: Animal evidence indicates that some consolidated memories when reactivated (retrieved) need to be reconsolidated. During this process, memories can be enhanced or weakened. Targeting reconsolidation of traumatic memories has been proposed as a treatment for post-traumatic stress disorder (PTSD). This presentation will recap a "proof of concept" study presented as a poster at ACNP 2005 and then go on present new data from pilot PTSD patients treated with this experimental therapeutic modality.
Methods: In the "proof-of-concept" study (Brunet et al, 2005), we tested whether post-reactivation administration of the beta-adrenergic blocker propranolol, which reduces reconsolidation of aversive memories in rodents, would weaken the traumatic memory, or conditioned fear response, in persons with chronic PTSD, as manifest in decreased psychophysiologic responses while they subsequently imagined their traumatic event. Participants described their traumatic event during a script preparation session lasting approximately 15 to 30 minutes and thereafter received either randomized, double-blind, 40 mg short-acting propranolol followed two hours later by 60 mg long-acting propranolol (n=9), or look-alike placebo capsules (n=10). A week later, they engaged in script-driven mental imagery of their traumatic event while physiologic responses were recorded. In the treatment study, we have to date treated three pilot patients with chronic PTSD in the single-blind propranolol condition; data from additional patients are expected to be available at the time of the panel presentation. Each patient underwent a series of six weekly therapy sessions, each consisting of 10-15 minutes of abreaction of their traumatic experience, immediately followed by propranolol in the same dosage. Post- (vs. pre-) treatment score on the Clinician-Administered PTSD Scale (CAPS) served as the outcome measure.
Results: In the "proof-of-concept" study, physiologic responses during script-driven traumatic imagery were significantly smaller in the

participants who had received post-reactivation propranolol a week earlier. Multivariate analysis of variance yielded $F(3,15)=5.1$, $p=.007$, $\eta 2=.49$. Univariate analyses yielded: for heart rate, $t(17)=2.0$, $p=.03$, Cohen's $d=.92$; for skin conductance, $t(16)=2.6$, $p=.01$, $d=1.23$; for corrugator electromyogram, $t(15)=.6$, $p=.28$, Cohen's $d=.29$. In the pilot treatment study, mean CAPS score for the three pilot subjects declined from 57.3 to 32. Mean within-subject improvement was 54%.
Conclusion: The results of the "proof-of-concept" study are consistent with pharmacologic blockade of reconsolidation of traumatic memories in PTSD. This represents the first controlled study supporting the conclusion that targeting reconsolidation can improve an objective aspect of human psychopathology. In the pilot treatment study, the 54% mean improvement is about the same as achieved by a series of cognitive behavior therapy sessions for PTSD. However the total duration of exposure (followed by propranolol) with the new treatment was about 1 hr. (10 min. per session x 6 sessions), as opposed to the 15 or more hours that CBT would require. We do not suggest that results from three pilot subjects prove the efficacy of this novel treatment or its mechanism of action. We do suggest, however, that they are sufficiently encouraging to support the performance of a double-blind, placebo controlled study. Reconsolidation offers the possibility of capitalizing on cellular plasticity to reverse the neuroanatomic and neurophysiologic underpinnings of mental disorders.

Panel Session
The Neuroscience of Affiliation: From Basic Science to Experimental Therapeutics in Autism and Related Disorders

Neuropetides Associated with Social and Emotional Deficits in Rhesus Macaques

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Monkeys reared in pathogenic rearing conditions manifest considerable deficits in reciprocal social interaction, and increased self-directed behaviors. The pattern of behavioral changes has remarkable face validity for social deficit syndromes associated with some human psychopathology. We have been interested in the possibility that disruptions in normal social development in non-human primates might be expressed in neuropeptide systems which have emerged in rodent studies as important candidates for a unique social biology. We have compared the outcomes of differing rearing histories on social, emotional, endocrine and post mortem brain measures. In recent studies, we have described persistently reduced CSF OT levels in male rhesus monkeys with significant social deficits. We also found that OT levels were positively related to the expression of affiliative social behaviors. Alterations were also detected in both CRH and AVP receptor binding patterns in limbic structures likely to influence social and emotional development. Further studies suggest that these alterations may be associated with potential risk factors for adult psychopathology and sensitivity to therapeutic pharmacology. Taken together, these data suggest that abnormal rearing influences the development of brain systems critical to normal social and emotional competence in rhesus monkeys and may provide insights about the emergence of adult psychopathology.

Impact of Oxytocin on Circuitry for Social Cognition and Fear in Humans

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In animals, oxytocin is a key mediator of complex emotional and social behaviors, reduces anxiety and impacts on fear conditioning and extinction. Recently, oxytocin administration in humans was shown to increase trust, suggesting involvement of the amygdala, a central component of the neurocircuitry of fear and social cognition that has been linked to trust and highly expresses oxytocin receptors in many mammals. We report on functional neuroimaging and genetic studies in healthy human subjects. In males receiving oxytocin or placebo, oxytocin potently reduced activation of the amygdala and reduced coupling of the amygdala to brainstem regions implicated in autonomic and behavioral manifestations of fear. Using imaging genetics, we are characterizing the effects on brain structure and function of genetic variation in the vasopressin receptor 1A gene (AVPR1A) (RS1 and RS3 microsatellite repeats), implicated in animal models of social behavior and affiliation, as well as the oxytocin receptor gene (OXTR), implicated in risk for autism. Taken together, the results suggest neural mechanisms for the effects of oxytocin in social cognition in the human brain that implies potential therapeutic uses.

Oxytocin and Experimental Therapeutics in Autism Spectrum Disorders

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Background: Animal studies point to the role of oxytocin in regulating affiliative behaviors. Oxytocin is implicated in maternal and psychosexual behaviors in rats and sheep and pair-bond formation in prairie voles. Moreover, studies suggest that oxytocin facilitates social cognitive abilities crucial to social success: oxytocin facilitates social memory and oxytocin knockout mice show deficits in social memory. Finally, recent research suggests that oxytocin is involved in prosocial behavior in healthy humans. These findings have implications for clinical disorders involving social functioning deficits and/or disrupted attachment including autism, social phobia, and borderline personality disorder. We have been interested in applying these findings to the treatment of autism spectrum disorders. Given that social interaction deficits are a core feature of autism, and that oxytocin is involved in regulating affiliative behaviors, oxytocin may play a role in autism. In previous research we have found that intravenous administration of oxytocin reduces repetitive behaviors in autism, a core symptom domain of this disorder. The present investigations extend this research.
Methods: Study 1 investigates the effects of intravenous oxytocin on social cognition in autism. In this double-blind, randomized, cross-over study, adults with autism spectrum disorders underwent two challenge procedures in which they received oxytocin (Pitocin 10 IU/ml) or placebo infused over a 4-hour period. Comprehension of affective speech (happy, indifferent, angry, and sad) in neutral content sentences was assessed at baseline and throughout the infusion. Study 2 combines functional magnetic resonance imaging (fMRI) with the intravenous oxytocin administration to study the neurobiological effects of oxytocin on social cognition and repetitive behaviors in autism. In this double-blind, parallel design study, adults with autism spectrum disorders were randomly assigned to receive intravenous oxytocin (Pitocin 10 IU/ml) or placebo. Participants underwent an fMRI scan at baseline and during the infusion (from hours 3 to 4), while performing a task assessing face/emotion processing and controlled inhibition. Study 3 investigates intranasal oxytocin in the treatment of autism. Adults with a diagnosis of high-functioning autism or Asperger's Disorder were enrolled in a 6-week randomized placebo controlled trial. Intranasal oxytocin (24 IU) or placebo was administered twice daily. Outcome measures included mood, anxiety, irritability and global functioning as well as repetitive behaviors and social information processing and social functioning.
Results: Study 1: All participants showed improvements in affective speech comprehension from pre- to post-infusion; however, whereas those who received placebo first tended to revert to baseline after a delay, those who received oxytocin first retained the ability to accurately assign emotional significance to speech intonation. Study 2: Preliminary data indicates increased activation of the fusiform face area during the face-processing component and increased activation of the prefrontal cortex during the response inhibition component from pre- to post-oxytocin infusion. Study 3: Result from the intranasal oxytocin versus placebo treatment study on social information processing, social functioning and repetitive behaviors will be presented.

Conclusion: These findings are consistent with studies linking oxytocin to social recognition in rodents as well as studies linking oxytocin to prosocial behavior in humans, and suggest that oxytocin has therapeutic potential for the treatment of clinical disorders involving social functioning deficits such as autism.

Effects of Oxytocin on Social Phobia

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Social phobia is an anxiety disorder characterized by clinically significant anxiety reactions and extreme discomfort occurring in anticipation of or following exposure to social settings including performance and test situations. Social phobia has major public health significance and ranks as the third most common mental health disorder after depression and alcoholism. We still know very little about the etiology of this disorder, and how it can be effectively treated. Evidence for the key role of the neuropeptide oxytocin in prosocial behavior, affiliation, stress, and anxiety has come primarily from studies in animals. In humans, we were recently able to show that oxytocin improves trust and protective effects of social interaction on neuroendocrine responses to social stress. Here, we examined the effects of a pharmacological manipulation of the central availability of oxytocin and the resulting altered ability to use social support on psychosocial and physiological reactivity to socio-evaluative stress exposure in patients with social phobia. Sixty male patients who fulfilled DSM-IV criteria for a diagnosis of generalized social phobia and 60 age-matched male healthy control subjects were exposed to a standardized socio-evaluative stressor (Trier Social Stress Test), which primarily consists of an unprepared speech and mental arithmetic task performed in front of an audience. In a placebo-controlled double-blind design, all participants were randomly assigned to receive intranasal oxytocin (24 IU) or placebo 45 min before stress, and either no social support or social support from their partner during the preparation phase. Stress responsiveness to the socially phobic situation was assessed by psychometric parameters (visual analog scales, questionnaires), endocrine parameters (cortisol, adrenocorticotropin, norepinephrine, epinephrine, oxytocin, prolactin), and an autonomic nervous system parameter (heart rate monitor). Three-way analyses of variance with repeated measurement revealed the expected increase in anxiety, physical arousal, and avoidance in the total group of patients (all $p < .001$). No significant differences in anxiety rating before social stress exposure were observed among groups. There was a significant attenuating effect of oxytocin treatment on physical discomfort (oxytocin x time effect, $p < .001$) . Importantly, a significant interaction effect (oxytocin x social support x time) was obtained, with the lowest anxiety levels ($p < .01$) and lowest physical arousal levels ($p < .01$) in patients who received both oxytocin and social support. Oxytocin seems to enhance the patient's ability to socially interact, thereby reducing anxiety and physical discomfort. Considering the large number of patients suffering from social phobia, these findings may have clinical implications for the development of new therapeutic strategies for the treatment of anxiety disorders.

Panel Session
Unravelling Sources of Genetic Complexity of Complex Disorders: Lessons from Neurologic Disorders

Contribution of Genetic Epidemiologic Approaches to Complex Disease Etiology

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This presentation will provide the context for this symposium regarding the sources of complexity in neurospsychiatric disorders in terms of design and methodologic issues. The increased availability of population based samples of cases and controls along with genotypes will present new challenges in analysis and interpretation. Steps to maximize these newly available tools will be discussed. Topics reviewed will include: the importance of systematic ascertainment, population-wide sampling, selection of appropriate controls, the use of extended family studies to maximize a priori knowledge regarding genotypic relative risk, and analytic methods matched to disease characteristics and risk factors. An overview of the status of current knowledge regarding the role of genetic factors in the etiology of neurologic disorders and specific issues relevant to Alzheimer's disease, narcolepsy and epilepsy will be summarized. More extensive data on the current status of genetic studies of migraine and sources of complexity including phenotypic heterogeneity, sex- and age-dependent expression and comorbidity will be described. These phenomena will be illustrated in data from our family and twin studies on the specificity of the components of migraine as well as co-occurrence of migraine and comorbid disorders. Strategies for future studies to maximize the probability of identifying genetic factors underlying complex neuropsychiatric diseases will be described.

Narcolepsy: From Animal Models to the Human Disorder

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This presentation will illustrate the use of animal models to study diseases with a complex human genetic basis, using the example of narcolepsy. Narcolepsy is genetically complex, and environmentally influenced. One of the predisposing factors is the Human Leukocyte Antigen (HLA) DQ, and at this locus multiple alleles interact to confer various degrees of susceptibility. An autoimmune mediation is suspected, but not yet proven. Positional cloning in a canine single gene mutant model and mouse knock out studies have demonstrated that the key pathway involved in the pathophysiology of the disorder is the neurotransmitter hypocretin (orexin). A deficiency in this system mediates the symptoms of the disorder in humans, but not through direct mutations of these genes. Gene expression studies in human brains and rodent narcolepsy models have led to the identification of other candidate molecules with preferential expression in hypocretin cells. These molecules are novel candidates for the pathophysiology of narcolepsy, either as targets for a putative autoimmune attack or as mediators of hypocretin cellular death. Zebrafish are also being used in a functional knock down model for some of these candidate genes and to study hypocretin cell physiology. The zebrafish model can also be used to screen for novel mutations that may affect hypocretin cell development and maintenance. Together with more classical genome-wide association studies, work in animal models is likely to assist in the discovery and functional testing of novel narcolepsy susceptibility factors.

Genome Wide Studies: Analyzing Data Beyond Association and Interpreting Negative Data

John Hardy*

Laboratory of Neurogenetics, NIA, NIH, Bethesda, MD, USA

The release of high throughput genotyping platforms and the (relatively) cheap costs of genotyping across the HapMap means that genome wide studies of common disease are now possible. Clearly, these studies have the potential to identify risk factor alleles by association, but what is not appreciated is that they also generate data which allow pathogenic alleles through both homozygosity mapping and by identifying insertions and deletions. I will discuss these applications with respect to our ongoing whole genome studies in Parkinson's disease, stroke, ALS and Alzheimer's disease. Another, perhaps less appreciated, benefit of whole genome studies is that negative data allows researchers to eliminate hypotheses of pathogenesis: thus, if an adequately powered study is designed and executed, and fails to find a

risk locus, this allows prevalent hypotheses of pathogenesis to be discarded, and incremental progress to be made.

Pharmacogenetics – Lessons from Anti-Epileptic Drugs
David Goldstein*

IGSP Center for Population Genomics & Pharmacogenetics, Duke Univ Medical Center, Durham, NC, USA

Many clinical challenges remain in the treatment of epilepsy, including the optimization of dosing, the occurrence and management of adverse reactions, and perhaps most importantly, the significant minority of patients who do not respond well to pharmacological treatment. The potential modes of action of most anti-epileptic drugs are known, permitting detailed candidate gene and candidate pathway screens for gene variants that may influence response. Such variants would be of potential relevance in the treatment of epilepsy and other in other indications that make use of anti-epileptic drugs. Here I report the results of a search for genetic determinants of predisposition to epilepsy and response to anti epileptic drugs in a data set of 3000 SNPs drawn from 300 candidate genes related to the action of anti-epileptic drugs.

Study Group Session

Research with Prisoners: Ethics and Opportunities
Wilson M. Compton*, Charles P. O'Brien, Kent A. Kiehl, Robert Schwartz, Jeffrey L. Metzner, Nora D. Volkow and Larry Palmer

National Institute on Drug Abuse, Bethesda, MD, USA

Despite great need for improved treatment of prisoners, restrictions on research among incarcerated persons have significantly limited progress. The proposed Study Group will address this topic in a round-table format with expert participants from key areas: psychopharmacology, neuroscience, public health, ethics and the law. Of note, the Institute of Medicine (IOM) currently is finishing a report on "Ethical Considerations for Revisions to the DHHS Regulations for Protection of Prisoners Involved in Research". The IOM report will be released during the summer or fall of 2006, and discussion of the results will be timely and important for ACNP. Prisons and jails provide settings of immense need for psychopharmacology services. In the United States, 2.1 million persons were incarcerated at the end of 2004 (Bureau of Justice Statistics, 2005), among whom up to 75-80% may be in need of addiction treatment (ONDCP, 2001). In addition, approximately 1 in 7 incarcerated persons have serious mental illness (Bureau of Justice Statistics, 1999). Thus, criminal justice settings are ripe for psychopharmacological intervention to address addiction, serious mental illness, and personality disorders. Research must fill this gap. Despite the well-documented need for treatment—especially innovative treatments that may be more effective or efficient than current standards of care, little research is conducted in criminal justice settings. The reasons for this lack of research are multiple, but a primary reason is the barrier to research among prisoners based on ethical concerns. A key issue is whether and how the ethical bases for research with prisoners differ from those for research with non-prisoners. In particular, what are the specific considerations or safeguards necessary to ensure that research with prisoners is conducted ethically, while also encouraging development of innovative approaches to address the multiple needs of the population? To promote ACNP discussion of and participation in the topic of research in prisoner populations, this study group will bring together a panel of experts from psychopharmacology (Drs. O'Brien and Schwartz), public health (Dr. Compton), clinical neuroscience (Drs. Kiehl and Volkow), ethics (Dr. Moreno) and the legal system (Drs. Litton and Metzner) to discuss the problems in working within the prison setting and to review plans for allowing the conduct of ethical, useful and important psychopharmacology research within this population. Of note, Drs. Moreno and Metzner served on the IOM panel, and so their participation will ensure that the key issues raised in the IOM report will be addressed. The goal will be to review concepts that might be developed into a position statement for ACNP to consider issuing. In particular, the group will review ways to encourage research within criminal justice settings while maintaining the highest ethical standards. The ultimate purpose is to speed the development of effective, useful and feasible interventions for this vulnerable and needy population.

Study Group Session

Comparing the Cost- Utility of Psychopharmacological vs. Psychosocial Treatments for Schizophrenia: Why and How
Daniel J. Luchins*, Patricia Hanrahan, Robert Rosenheck, and Ramy Mahmoud

Psychiatry, University of Chicago, Chicago, IL, USA

Appropriate treatment for persons with schizophrenia requires both psychotropic medications and psychosocial treatment. In the past, treatment with typical antipsychotics was both better supported by empirical evidence and far less costly than available psychosocial treatments. Not surprisingly, given limited resources, payers chose to fund medications. However, there is now extensive empirical support for several "Evidence Based Treatments" (Assertive Community Treatment, Supported Employment, Family Psychoeducation, etc) which are comparably priced with the new, more expensive, atypical antipsychotics. In the context of maximizing limited resources it is therefore necessary to ask the question: "How do you measure and compare the utility of these very different interventions." The session will provide an opportunity to address this issue by bringing together discussants from different disciplines and very different perspectives. 1)Daniel Luchins, MD (Chairman) will discuss, from the perspective of his experience as the Clinical Director of a state mental health authority, the rational for these comparisons and research methods to carry them out such as contingent valuation (CV) or naturalistic trials through Medicaid waivers. 2)Patricia Hanrahan, PhD will discuss, from the perspective of her experience as Chief of Clinical Evaluation for a state mental health authority, the cost-utility of current Evidence Based Treatments for schizophrenia. 3)Robert Rosenheck, MD, an academic and VA evaluator with extensive experience in evaluating both psychosocial and psychopharmacolgical interventions (including the pharmacoeconomic analysis of the CATIE Study), will discuss this research including both methodological and policy implications 4)Ramy Mahmoud MD will discuss, from his perspective as Vice President for Medical Affairs at a major pharmaceutical corporation and lead author of the Risperidone Outcome Study of Effectiveness(ROSE), practical and methodological issues with such comparisons.

Study Group Session

Identifying and Characterizing Drug-Induced Risks: Can We Improve upon Current Strategies for Assessing Drug Safety?
Donald S. Robinson*, Paul Leber, Arif Khan, Tamar Wohlfarth, Robert M. Hamer and Donald F. Klein

Worldwide Drug Development, Burlington, VT, USA

Pharmaceutical sponsors, U.S. and European regulatory agencies, and the general public are, not withstanding the existence of elaborate regulatory systems for the pre-market clearance of new drugs, still concerned with the capacity of drugs to cause injury. This forum will consider the limitations and shortcomings of strategies currently employed to identify and characterize risks reported to be associated with the use of psychotropic drugs. The subject is of considerable interest because publicity given to repeated reports of previously unrecognized drug-attributed risks has seemingly undermined public confidence in the safety of the drug supply. The study group will consider

whether the public concern about the safety of drugs is warranted, and, if so, what steps might be taken to improve how drug-associated risks are identified, characterized, and evaluated, both during premarket development and post-approval. The study group will first examine how FDA strategies for collection and analysis of evidence bearing on drug-associated risks for new agents have evolved in recent decades, and consider why, despite modifications, important risks of new drugs may still go undetected at time of their approval. Concerning marketed drugs, the panel will consider methods now in use to monitor safety and to assess the importance of events reported in association with their use. Strategies that may improve detection of drug-induced risk will be discussed, among them, national drug registries, use of large cross-linked, computerized medical databases in the U.S. (e.g., HMOs, VA, DOD), and other meta-analytic approaches that might be applicable to assessing the causality of drug-associated adverse events. The ACNP Task Force on Clinical Trials currently is exploring the potential for utilizing large databases for ongoing monitoring of the safety of marketed psychotropic agents as well as for gauging the effects of safety-related events on psychopharmacologic treatments and mental health care delivery. Methodological limitations and statistical considerations with regard to each of these approaches to drug safety assessment will be considered.

Study Group Session

Fostering Collaborations Across Academia, Industry, and Government to Develop Biomarkers for Decision-Making in Drug Development

Dean F. Wong*, John Csernansky, Rikki Waterhouse, Don Burns, Linda Brady, Kalpana Merchant and Constantine Lyketsos

Radiology, Johns Hopkins University, Baltimore, MD, USA

Biomarkers can play a critical role in drug development for dose ranging, assessing toxicology/safety, and characterizing mechanisms of drug action, even when there is limited information about the pathophysiology of the targeted disease. In this study group, the participants will address policy issues related to fostering collaborations amongst across and within Pharma, government and universities to promote the development of biomarkers for CNS drug development. This workshop has been developed by the Liaison Committee of the ACNP. In the first part of the study group, policy issues related to the development of novel radiotracer biomarkers will be discussed. The focus of this discussion will be the results and implications of a proposal to develop a clearinghouse mechanism to facilitate development and sharing of radiotracer biomarkers to facilitate drug development. The proposal came out of a meeting of liaison committee members supplemented by legal and R&D participants from pharmaceutical companies, researchers and tech transfer participants from academia and government held on April 5, 2006, sponsored by the ACNP Liaison Committee. The clearinghouse (CH) concept was put forward as a unique collaborative structure for promoting the development of novel radiotracers - 4 hypothetical case studies involving a CH will be discussed, including the sharing of highly confidential radiotracers amongst Pharma and Academia centers, the sharing of publicly disclosed radioligands, and the possibility of pre-competitive collaborations to design new radiotracers existing targets. The project grew out of previous workshops on radiotracer development held at ACNP. This workshop will address how the proposed development model will work. Discussants will include Dean Wong (JHU), Don Burns (Merck), Rikki Waterhouse (Columbia) and Linda Brady (NIH). Dialogue by all interested PET research centers and Pharma will be strongly encouraged and incorporated into Committee meetings. In the second part of the study group, the need for new biomarkers to facilitate drug development for Alzheimer's disease and other disorders of cognition will be discussed. The focus will be divided into a discussion of structural and functional neuroimaging biomarkers, and a discussion of non-brain biomarkers ascertained in blood, CSF, or urine. Biomarkers of factors that might serve to subtype neurodegenerative diseases, assess disease state or disease progression, and characterize the distribution activity of a pro-cognitive drug will be discussed. Specific approaches for sharing such development perhaps in analogy to some cases using the radiotracer model will be addressed and integrated with those speakers. Discussants for this section will include John Csernansky (WU), Constantine Lyketsos (JHU), and Kalpana Merchant (Lilly) as well as the radiotracer consortium group above.

Study Group Session

Comparative Effectiveness of Antipsychotic Drugs: Complete Results of the CATIE Study

Jeffrey Lieberman*, Scott Stroup, W. Wolfgang Fleischhacker, Shon Lewis, Martin Swartz, Richard Keefe and Susan Essock

NY State Psychiatric Institute, Columbia University, New York, NY, USA

This study was designed to determine the comparative effectiveness of the first and second generation antipsychotics and of the SGAs to each other. 1500 patients were randomized to controlled treatment under blind conditions and followed for up to 18 months. If treatments were ineffective or intolerable patients could be rerandomized to additional drugs. Outcome was measured in terms of efficacy, safety and cost. This study group will present the complete results of the CATIE study including new data on adherence, violence, substance abuse, quality of life and cost effectiveness. The data reveal the relative effectiveness of olanzapine, quetiapine, risperidone and ziprasidone to perphenazine; and clozapine to olanzapine, quetiapine, risperidone. The discussants will interpret these results in the context of similar effectiveness studies conducted in Europe. The study group will discuss the implications of these results for clinical practice and policy.

Tuesday, December 5, 2006

Panel Session
Cellular Mechanisms of Stress-Induced Atrophy of Prefrontal Cortex

Age-Dependent Cellular Changes in the Prefrontal Cortex in Postmortem Tissue from Depressed Subjects

Grazyna Rajkowska*

Psychiatry and Human Behavior, University Mississippi Medical Center, Jackson, MS, USA

Background: Recent postmortem studies in depression reveal prominent reductions in the density of glial cells and only subtle or no changes in neuronal pathology in fronto-limbic brain regions. In particular, we found age-dependent reductions in the density of astrocytes and GFAP-protein level in the dorsolateral prefrontal and orbitofrontal cortex in subjects with major depressive disorder. These glial reductions were significant in younger (<60 years old) depressed as compared to age-matched controls and older (>60) depressed. In contrast, prefrontal neurons were only mildly affected in younger depressed subjects exhibiting smaller cell body size and lower density of large-size neurons. Clinical evidence indicates that depression in elderly differs from that of younger patients by its etiology, phenomenology and cerebrovascular pathology. However, to date cell pathology has not been examined in older patients with major depressive disorder and has not been compared to that of younger depressed subjects. Therefore, the goal of the present study was to test whether changes in neuronal density in the prefrontal cortex are age-dependent.

Methods: Orbitofrontal cortex (Brodmann area 47) from 15 elderly subjects (>60 years old) with diagnosis of major depressive disorder and 11 age-matched non-psychiatric controls was analyzed using 3-D cell counting. The packing density of pyramidal (glutamatergic) and non-pyramidal (GABAergic) neurons and glial cells was measured. In addition, we compared neuronal density and size in elderly depressed subjects to that described previously in younger subjects (< 60 years old) with major depression.
Results: ANCOVA (with postmortem interval, tissue pH, time in formalin and age as co-variates) revealed that the overall (layers I-VI combined) density of orbitofrontal neurons was significantly reduced by 14% in elderly depressed subjects as compared to age-matched non-depressed controls. Further laminar analysis revealed that the density of pyramidal neurons was significantly reduced by 30-60% predominantly in layers III and V and to a lesser degree in layers II, IV and VI. Neuronal density was inversely correlated with age and reductions in neuronal density were more prominent in elderly than in younger depressed subjects. In contrast, neuronal soma size, density of non-pyramidal neurons and glial cell density in elderly depressed were comparable to those of age-matched control subjects.
Conclusions: Our data indicate that pyramidal neurons in the orbitofrontal cortex are selectively affected in elderly depressed subjects as compared to age-matched controls. This is in contrast to the findings from younger depressed subjects that mostly exhibit glial but not neuronal pathology in an analogous cortical region. This observation indicates an age-dependent progression of neuronal pathology in depression and differential cell pathology in young vs old subjects with major depression. It is possible that in younger depressed the factors resulting in cell pathology are different from those acting in elderly depressed. Genetic susceptibility, stress and a deficiency in neurotrophic factors may play a role in younger depressed whereas vascular lesions and neurodegenerative changes could contribute to cell pathology in elderly depressed.
Supported by: MH60451, MH61578, MH63187, MH67996, RR17701

Stress-Induced Structural Plasticity in Prefrontal Cortex, Amygdala and Hippocampus
Bruce S. McEwen*, Conor Liston and John H. Morrison

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The prefrontal cortex plays an important role in working memory and executive function and is also involved in extinction of learning, whereas the hippocampal formation is a malleable brain structure that is important for certain types of learning and memory. The amygdala is an important target of stress and mediates physiological and behavioral responses associated with fear and strong emotions. All 3 regions are targets of stress hormones and acute and chronic stress, and stress is known to precipitate and exacerbate mood disorders. New data indicates that the prefrontal cortex shows differential remodeling, with medial regions showing shrinkage and loss of synaptic connections and orbitofrontal cortex showing expansion of dendrites following repeated stress. These are accompanied by alterations in behaviors related to attention and executive function. Neuronal atrophy in the medial PFC parellel shrinkage of dendrites in the hippocampus, whereas dendritic expansion parallel increased dendrites in the basolateral amygdala, associated with increased anxiety and depression. In mPFC and hippocampus, these changes are largely reversible, whereas structural changes in amygdala appear to be longer lasting. In long-term depressive illness, the hippocampus and prefrontal cortex undergo atrophy, whereas the amygdala is hyperactive in anxiety and mood disorders and may undergo a biphasic change in structure – increasing in size in acute depression and shrinking on long-term depression. Yet, these are not necessarily "damaged" and may be treatable with the right medications. Supported by MH41256 and MH58911.

Stress-Related Dendritic Atrophy of Layer V Pyramidal Cells in Rat Medial Prefrontal Cortex: Association with Deficits in Excitatory Synaptic Transmission in Apical Dendritic Field
George K. Aghajanian* and Rongjian Liu

Psychiatry, Yale School of Medicine, New Haven, CT, USA

Background: A number of laboratories have reported that repeated immobilization stress leads to dendritic atrophy of layer II/III pyramidal cells in medial prefrontal cortex (mPFC), an effect mimicked by high levels of corticosterone. Interestingly, the atrophy seems to be confined to the apical dendritic field, with sparing of the basilar field. There have been no attempts previously to correlate these atrophic changes with electrophysiological measures. For a number of years we have studied the modulation of spontaneous excitatory synaptic currents (spEPSCs) in layer V pyramidal cells of rat mPFC brain slice by serotonin (via 5-HT2A receptor), hypocretin/orexin (via the hypocretin receptor 2), as well as other transmitters. These studies show that spEPSCs elicited by serotonin and hypocretin are generated primarily in the apical dendritic field. On that basis, we hypothesized that stress-induced atrophy of apical dendrites would result in attenuation of these electrophysiological responses.
Methods: Whole cell recordings were made from layer V neurons of the rat mPFC slice; the pipette solution contained the fluorescent dye Alexa 594 to label the recorded cells. Upon completion of the recordings, brain slices were fixed in formalin and mounted in DMSO (by method of Grace an Llinas) and then examined by 2-photon laser scanning which permits imaging at deep penetrations. Three-dimensional images for quantitative analysis were generated by Z-stacks of up to 200 scans at 1 micron steps.
Results: In control rats, these was a high correlation between the extent of apical dendritic branching and the frequency of spEPSCs induced by serotonin or hypocretin. In rats exposed to repeated brief restraint/immobilization stress (20 min/day) for 5-7 (but not 1 or 3 days) showed a mean reduction of ~20% in several measures of dendritic branching in the apical tuft region. These morphological changes were associated with reductions of similar magnitude in mean spEPSC responses to serotonin or hypocretin. In controls, when the distal apical dendrite (including tuft) was disconnected surgically, an even larger reduction in spEPSCs was seen (70-80%), indicating the importance of the apical dendritic field as site for their generation.
Discussion: These are the first studies showing electrophysiological correlates of morphological changes in response to stress in mPFC pyramidal cells. These results have several implications for mechanisms of stress-induced dendritic atrophy and associated electrophysiological deficits. Previous studies have shown that hypocretin induces spEPSCs predominantly at apical dendritic synapses formed by projections of the thalamic midline/intralaminar nuclei. Since these nuclei are essential for awareness and attention, an impairment of this ascending input could underlie certain stress-related cognitive deficits. Previous studies have also shown that serotonin-induced EPSCs are markedly depressed in conditional BDNF knockout mice. Since BDNF has been shown to be important for dendritic branching, this suggests a possible link between the mechanisms of stress-related dendritic atrophy and the deficits in serotonin response resulting from BDNF deficiency.

Chronic Stress Decreases the Proliferation of Glia in Prefrontal Cortex
Ronald S. Duman*, Gerald W. Valentine and Mounira Banasr

Psychiatry, Yale University School of Medicine, New Haven, CT, USA

One of the most consistent postmortem findings in depression is a decrease in the number of glia in prefrontal (PFC) and cingulate cortex of major depressive disorder (MDD) subjects (see Rajkowska, this panel). Decreased glia could contribute to the atrophy of neurons that is also observed and the altered function of PFC, including decreased cognition and attention (see McEwen and Aghajanian). To examine the cellular mechanisms underlying this decrease we have studied the influence of stress, a critical vulnerability factor for MDD, on the proliferation of non-neuronal cells in the PFC of rodents. We

find that exposure to repeated unpredictable stress for 15 days, but not shorter times, decreases the number of newborn cells in the PFC. Co-localization studies demonstrate that there is a reduction in the number of newborn cells that express markers of oligodendrocytes, which are reported to be decreased in MDD subjects, endothelial cells, and an unidentified population of cells. The reduction in newborn cells is reproduced by chronic administration of corticosterone, indicating that the effects of stress occur via elevated levels of this adrenal-glucocorticoid. We have also examined the influence of antidepressants, including a serotonin selective reuptake inhibitor (fluoxetine) and electroconvulsive seizure (ECS), on glial proliferation in the PFC. In contrast to the effect of stress, chronic antidepressant administration increases the proliferation of oligodendrocytes and endothelial cells in the PFC, and reverses the effects of chronic stress. We are currently examining the role of specific growth factors in the regulation of glial proliferation, and we are developing strategies for directly testing the impact of glia on the function of the PFC in behavioral models of depression.

Panel Session
Conducting Treatment Research in Decisionally Impaired Subjects: Problems and Possible Solutions

Disparities Between the Readability of Consent Forms and Educational Levels of Potential Subjects in Psychiatric Research

Paul S. Appelbaum*, Paul Christopher and Mary E. Foti

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Background: Discussions of informed consent in psychiatric research tend to focus on subject characteristics, such as the possibility of impaired decisional capacity. But the quality of consent depends on an interaction between subjects' abilities and the demands placed on them in the consent process. Poor readability of informed consent forms has been a persistent problem in clinical research, and the low educational attainments of many patients with mental illnesses suggest a still greater problem in psychiatric settings. To explore the extent of this potential disparity, we compared the readability of informed consent forms used in research approved by the institutional review board (IRB) of the Massachusetts Department of Mental Health (MA-DMH) with the highest grade level of education achieved by potential study subjects.
Methods: We calculated the readability (scored as grade level of education completed that is necessary to comprehend the text) of 154 informed consent forms using several standard formulas (Flesch Reading Ease Score, Flesch-Kincaid Grade Level, FOG Index, and Fry Graph). These data were compared with the maximum attained grade level of 12,848 potential adult participants in MA-DMH approved studies. Research studies were categorized by the reviewing IRB as reflecting one of three risk levels, and readability scores were stratified based on the risk level for each study.
Results: Approximately 35% of potential participants had not graduated high school, 37% had graduated from high school or obtained a GED, and 28% had some education beyond the 12th grade. The mean readability scores for the informed consent forms ranged between a grade level of 12 and 14.5, depending on the test used. On the Fry graph, for example, 10 forms fell beyond the 16th grade level, reflecting a performance demand at the graduate school level. The mean rating for the remaining forms was a 14th grade (i.e., college sophomore) reading level. Furthermore, the mean readability scores on all the measures increased with increasing risk level, indicating that those consent forms used in higher risk studies were, on average, significantly harder to comprehend.
Discussion: Although consent forms are just one way in which information is communicated to potential research subjects, they can play an important role in the informed consent process. These data suggest that the mismatch between the level of reading ability required to comprehend the forms and the mean educational level of potential subjects in a public mental health system all but doomed the possibility that subjects would glean useful information from the consent forms. Methods of reducing the complexity of consent forms, as part of improving the overall consent process, are much needed, and institutional review boards have a crucial role to play in facilitating this process. Though IRBs pay inordinate attention to consent forms in their review process, these data indicate that the result is not always a form that is in any way useful to potential research subjects.

Multimedia Consent for Research in People with Schizophrenia

Dilip V. Jeste*

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Studies suggest that decision-making capacity tends to be more impaired among persons with schizophrenia than in normal subjects, although considerable variability exists in this regard. It is important to study methods for enhancing consent procedures for treatment research. The goal of our ongoing investigation is to evaluate the usefulness of an innovative method of providing informed consent to middle-aged and older persons with schizophrenia. This is a randomized, controlled trial of two procedures for providing informed consent to a hypothetical protocol: a DVD-based, multimedia consent procedure incorporating audio, video, still pictures, motion pictures, graphics, animation, and text; and a routine consent procedure. The hypothetical protocol tested is a randomized, double-blind, placebo-controlled trial of a cognitive-enhancing drug with some potentially serious adverse effects. The primary outcome measure is decision-making capacity. The study sample is comprised of subjects with schizophrenia or schizoaffective disorder who are older than 40 years of age. Preliminary results suggest that the patients who received the DVD consent had better comprehension of the consent than those who received routine consent. A multimedia consent is thus feasible, and may be associated with better comprehension of consent material.

Influences on Proxy Decision Making for Alzheimer's Disease Research

Laura B. Dunn*

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Background: Informed consent raises ethical challenges in Alzheimer's Disease (AD) protocols, and proxy consent plays a central role in the ethical integrity of this research. Few studies have examined influences on proxies' decision-making processes regarding AD research. Given an unclear legal and regulatory framework for proxy consent for research with cognitively impaired people, there is a clear need for empirical evidence focused on this important ethical aspect of research decision making.
Methods: 63 proxy decision makers (43 spouses, 15 adult children, and 5 other; mean age 68.8 years, SD 12.3; range 38-94 years) for people with AD were randomly assigned to receive informed consent for one of three hypothetical clinical research protocols, ranging in type and level of described potential risk and potential for direct benefit. The protocols were presented using realistic consent forms, written to resemble real-life AD protocols. These ranged from least risky (MRI/Behavioral) to intermediate risk (Pharmacologic study) to most risky (Vaccine study). Proxies were asked to rate how risky (from 1="Not risky at all" to 5="Very risky") they believed the study would be to their relative, as well as their willingness to enroll their relative in the protocol (also using a 5-point scale, with 5="Very willing"). Five other, briefly-described research procedures were presented as well. Proxies were also asked about their views about whether they themselves should be able to make proxy decisions with respect to four general categories of studies, ranging from minimal risk to potentially serious risks (with and without the potential for direct benefit), using four response categories, from "Definitely not" to "Definitely yes"). In a smaller set of proxies (n=15), we assessed agreement with two statements regarding the use of two models of decision-making—based on what they believed their relative would want ("substituted judgment"), and

based on what they believed was in their relative's best interests ("best interests").
Results: Proxies viewed the objectively highest risk:benefit ratio protocol (the Vaccine study) as carrying moderate risk, with a mean rating of 3.38 (SD 1.24) out of a possible 5 points; the Pharmacologic study had a mean risk rating of 2.89 (SD 1.70) while the MRI/Behavioral protocol was perceived as the least risky (mean 1.22, SD 0.67). These ratings differed significantly across protocols ($p<0.001$). Higher general interest in enrolling one's relative in research was associated with lower risk ratings ($r=-.334$, $p=0.008$). Proxies' willingness to enroll their relative also differed across the three protocols ($p=0.048$), and was inversely associated with perceived risk level ($r=-.513$, $p<0.001$), and positively associated with both proxies' perception of the patient's interest in enrolling ($r=.602$, $p<0.001$) and proxies' overall interest in enrolling their relative in research ($r=.718$, $p<0.001$). Mean ratings regarding proxies' belief about the appropriateness of making the decision on behalf of the AD patient were in the "Probably Yes" to "Definitely Yes" range (3.13 to 3.44 out of 4) for all four protocols.
Discussion: Proxies consider a combination of factors in making research decisions on behalf of the AD patient, including perception of risk and benefit, and a combination of substituted judgment and best interests perspectives. These findings suggest a need for further empirical research that carefully examines proxies' decision-making processes and behavior; such research will help move the field forward in constructing ethically and empirically informed frameworks for proxy consent for research involving decisionally impaired individuals.

Decisional Competence to Complete Psychiatric Advance Directives

Eric B. Elbogen*, Jeffrey Swanson, Paul Appelbaum and Marvin Swartz

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Background: Psychiatric advance directives (PADs) allow people to declare preferences for future mental health treatment in the event of a psychiatric crisis. In PADs, now in 22 states, patients can provide advance consent or refusal of psychotropic medications (including experimental drugs) and information about medical disorders and medication side effects. Patients must be competent to complete PADs for the documents to be valid, but little is known about this decisional capacity or about how competence can be improved.
Method: At baseline, N=469 adults with schizophrenia, schizoaffective disorder, bipolar disorder, or depression with psychotic features were administered the Decisional Competence Assessment Tool for Psychiatric Advance Directives (DCAT-PAD), based on the conceptual model used by the MacArthur Competency Assessment Tool (MacCAT-T). "Competence to write a PAD" was measured by evaluating the ability to understand PADs, appreciate whether PADs are relevant, and reason how PADs may affect their lives. "Competence to make treatment choices within the PAD" used hospitalization as the index treatment and was measured by evaluating the ability to understand hospitalization, appreciate whether the hospital may be relevant, and reason how hospitalization may affect their lives. After the baseline interview, subjects were randomized to a 'usual-care' control group or a Facilitated PAD (FPAD) intervention group in which they met one-on-one with a trained facilitator to create a PAD. If participants wished to prepare a PAD, the facilitator assisted in doing so by (1) eliciting preferences and advance consent/refusal for psychotropic medications, hospital treatment, or ECT; and (2) gathering information about medical conditions, crisis symptoms, relapse and protective factors, and strategies to avoid use of seclusion and restraints. At one-month, the DCAT-PAD was re-administered.
Results: Baseline DCAT-PAD domains were regressed onto demographic, clinical, and neuropsychological variables using multivariate statistics. There was some overlap in "competence to write a PAD" and "competence to make treatment decisions within a PAD." For both, understanding was predicted by abstract thinking, verbal memory, and IQ, appreciation was predicted by psychiatric symptoms, and reasoning was predicted by insight into disorder. But the appreciation and reasoning domains for "competence to write a PAD" were also predicted by IQ. Each predictor is significant at a $p<.01$ level. To analyze effects of randomization, we compared baseline and one-month scores on the DCAT-PAD domains employing repeated-measures ANOVA. Subjects in the intervention group showed significantly higher improvement in the reasoning domains for "competence to write a PAD"($F=8.65$, $df=1$, $p=0.003$) and "competence to make treatment decisions within a PAD"($F=4.30$, $df=1$, $p=.03$). Of note, intervention and control subjects above the median IQ of 100 did not differ in reasoning scores at one month whereas subjects below the median IQ in the intervention group showed significantly greater improvement in the reasoning domains compared to their counterparts in the control group.
Discussion: The data reveal cognition and symptoms relate patients' competence to complete PADs. The results show an intervention to facilitate PADs improved patients' competency to complete PADs. The findings imply for more impaired clients, a facilitation of PADs may be helpful, or even necessary, to augment decisional capacity to a level that ensures the PAD is valid and followed by clinicians. Such steps may be needed if PAD laws help people with psychotic disorders communicate clinical and medical information during psychiatric crises.

Panel Session
Genetics of Cognitive Deficits in Schizophrenia: What Is Inherited and How?

Intellectual Functioning in Schizophrenia Patients, Their Unaffected Parents, Siblings, and Controls: A Population Based Study

Mark Weiser*, Abraham Reichenberg, Jeremy M. Silverman, Asaf Caspi, Gad Lubin, Mordechai Shmushkevitch, Philip D. Harvey, David A. Greenberg and Michael Davidson

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Background: Many aspects of cognition are impaired in patients with schizophrenia. These impairments are familial and appear to account for much of the psychosocial disability associated with the illness.
Methods: We conducted a historical population-based cohort study. The cohort included some 480,000 siblings born in Israel and assessed by the Draft Board over 15 consecutive years. Cohort members were followed up for schizophrenia using a national psychiatric hospitalization case registry. The merger identified 1,753 cases with schizophrenia, first hospitalized at least one year after assessment. Cases had 3,286 never-hospitalized siblings. We also identified 350 of the parents of these patients, and for each parent selected 10 controls matched for age and gender.
Results: The cognitive test scores of future patients were lower (poorer functioning) then their siblings, who, in turn had lower test scores then control siblings ($p<0.0001$). Parents of siblings had poorer test scores compared with controls. Data will be presented on the actual scores of the patients and on expected cognitive scores based on their parents scores.
Conclusions: Cognitive deficits are probably associated with genetic liability to schizophrenia. Such measures could be utilized as intermediate phenotypes in genetic studies of schizophrenia.

Gray and White Matter Volume Abnormalities in Monozygotic and Same-Gender Dizygotic Twins Discordant for Schizophrenia: A Five Year Follow-Up Study

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Background: Whole brain tissue volume decreases in schizophrenia have been related to both genetic risk factors and disease-related (possibly nongenetic) factors; however, whether genetic and environmental risk factors in the brains of patients with schizophrenia change over time and whether these changes are related to (genetic) risk or illness is unknown.

Methods: Magnetic resonance imaging (1.5 T) brain scans of 13 monozygotic and 14 same-gender dizygotic twin pairs discordant for schizophrenia were acquired and compared with 13 monozygotic and 14 same-gender dizygotic healthy control twin pairs. Scans were repeated after a five year interval.
Results: Repeated-measures volume analysis of covariance revealed decreased whole brain volume in the patients with schizophrenia as compared with their co-twins and with healthy twin pairs. Decreased white matter volume was found in discordant twin pairs compared with healthy twin pairs, particularly in the monozygotic twin pairs. A decrease in gray matter was found in the patients compared with their co-twins and compared with the healthy twins. Follow-up results will be presented.

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

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Background: The exploration of the genetic architecture of specific endophenotypes is a powerful strategy for understanding the genetics of schizophrenia versus searching for genes related to the "fuzzy" clinical endophenotype of DSM schizophrenia. The Consortium on the Genetics of Schizophrenia (COGS) has undertaken a large 7-site study to characterize the genetic architecture of some key endophenotypic measures. If available, DNA based analyses will be presented based on 5 CM whole genome scan from the summer 2006 CIDR DNA release.
Methods: Families consisted of both parents and at least 2 siblings discordant for schizophrenia. The COGS assesses the effortful, controlled processing endophenotypes of the Antisaccade Task, the Continuous Performance Test (CPT), the California Verbal Learning Task (CVLT), the Letter-Number Span (LNS), and several domains of the Pennsylvania Neuropsychological Battery. In addition, P50 suppression and prepulse inhibition data will be presented as "automatic" neurophysiological measures. At the time of the initial analyses of data (Spring 2006), 106 schizophrenic probands and their family members (N=411) had been assessed for heritability of endophenotypes. We expect to have data on almost 300 probands by December 2007. SOLAR was used to assess the heritability of each endophenotype, as well as the crucial important environmental and genetic correlations between the endophenotypes. In addition, heritability estimates for P50 suppression and PPI will be presented emphasizing the normalizing effects of atypical antipsychotics. Relevant and model data from strain-related rodent studies will also be used to discuss candidate genes.
Results: The volitional measures consisting of the Antisaccade Task, the LNS, the CPT, the CVLT and three domains from the Penn Battery were found to be significantly heritable (with h2r's ranging from 0.169 and 0.547 and p's <.05 to .001) with heritabilities ranging from 17 to 55%. Significant environmental and genetic correlations were also observed between many of the endophenotypic measures, providing some evidence for pleiotropy.
Conclusion: This is the first large-scale multi-site family-based heritability study of a large collection of endophenotypes for schizophrenia and suggests that neurocognitive, "volitional" endophenotypes will be important measures, in addition to automatic processing of endophenotypes, in determining the genetic basis of schizophrenia.

Genetic Prediction of a Schizophrenia Intermediate Phenotype Related to Cortical Information Processing

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Introduction: Schizophrenia susceptibility genes presumably impact on the development and plasticity of cortical systems involved in the expression of schizophrenia symptoms (Harrison and Weinberger Mol Psychiatry 2005). Since genes do not encode for hallucinations and delusions, carriers of susceptibility alleles/genotypes, (e.g. discordant MZ twins, unaffected siblings) express these genes as intermediate phenotypes if they are penetrant. Studies in discordant MZ twins (e.g. Goldberg et al Arch Gen Psychiatry 1990, Cannon et al AJHG 2000) indicate that concordance for cognitive deficits involving especially executive functions and working memory are greater than concordance for psychotic symptoms. These findings indicate that genetic risk for schizophrenia is expressed with greater penetrance at the level of intermediate phenotypes related to cortical cognitive processing. Such observations lead to the prediction that the effect size of risk genotypes for schizophrenia will be greater for cognitive processes related to schizophrenia than for the clinical diagnosis. We have reported a series of studies confirming this prediction for several putative schizophrenia susceptibility genes (e.g. COMT [Egan el al PNAS 2001], GRM3 [Egan et al PNAS 2004], DISC1 Callicott et al PNAS 20005). In this presentation, we have explored this prediction in the context of several other genes implicated in risk for schizophrenia.
Methods: fMRI was performed varying subsets of a large sample (N > 160) of carefully screened Caucasian normal subjects during performance of the N back working memory test. During this paradigm prefrontal processing is inefficient in patients with schizophrenia who perform the task well, and a qualitatively similar pattern is seen in their healthy siblings (Callicott et al AJP 2003). This pattern is thus representative of an intermediate brain phenotype related to increased risk of schizophrenia. The normal sample was genotyped for SNPs in CAPON, RGS4, Akt1, GAD1, and G72 that have shown positive association with schizophrenia in the literature, None of these genes showed consistent positive association in the CBDB sibling study family or case control samples. Genotype groups were carefully matched for age, gender, years of education, Wechsler Adult Intelligence Scale (WAIS-IQ) and task performance (accuracy and reaction time). Genotype groups (typically greater than 40 subjects in any group) were also matched for 100 unlinked SNPs across the genome to control for population stratification.
Results: For all of the genes typed, risk genotypes were associated with relatively inefficient BOLD responses measured with fMRI in the normal control samples. Control SNPs within the typed genes, i.e. SNPs not in LD with risk SNPs and not positive in earlier studies, were invariably negative.
Conclusion: These data confirm that risk SNPs in candidate susceptibility genes show greater effects on a phenotype based on cortical processing of executive cognitive information than on risk for schizophrenia per se.

Panel Session
Insulin Signaling in the Forebrain and Its Relevance to Cognition and Schizophrenia

Differential Regulation of AMPA and GABAA Receptors by Brain Insulin

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Both insulin and its receptors are expressed in the central nervous system (CNS) at high levels, and particularly associated with neurons. Since the glucose utilization in neurons is largely insulin independent, brain insulin has been suspected to have neuronal functioning distinct from metabolic regulations by its peripheral counterparts. Consistent with this idea, evidence has been accumulating in the past decade which strongly suggests that brain insulin may have critical roles in mediating both brain functions and dysfunction through regulating the function

and postsynaptic plasma membrane expression of glutamate and GABA-A receptors, the two principal neurotransmitter receptors which mediate excitatory and inhibitory synaptic transmission, respectively, in the mammalian brain. Using a combination of EM and electrophysiological techniques we have recently demonstrated that transient exposure of insulin to hippocampal neurons produces a rapid recruitment of GABA-A receptors from intracellular compartments to postsynaptic membrane surfaces, resulting in a potentiation of GABA-A receptor-mediated synaptic inhibition. Molecular characterizations further reveal that this increase in the number of postsynaptic GABA-A receptors is primarily mediated by the increased plasma membrane insertion of the receptors as a result of Akt-phosphorylation of the beta2 subunit of the receptor. In great contrast, a brief treatment of these neurons with insulin produces a long-lasting depression of synaptic transmission at glutamatergic synapses as a result of facilitation of clathrin-dependent endocytosis of AMPA type of glutamate receptors at the postsynaptic membrane. The facilitated AMPA receptor endocytosis requires src tyrosine phosphorylation of the GluR2 subunit of AMPA receptors. Compromised insulin modulation of either AMPA or GABA-A receptors appears to contribute at least in part to some of the brain dysfunctions associated with ischemia and brain insulin resistance. Thus, our results strongly suggest that in addition to regulating glucose metabolism, insulin may have critical roles in mediating neuronal functioning in the brain, and the differential modulation of AMPA and GABA-A receptors, the two principal neurotransmitter receptors in the brain, may be the primary mechanisms by which insulin exerts its non-metabolic neuronal modulations, thereby contributing to both brain function and dysfunction.

Genes Decreased in Schizophrenia Are Increased in SH-SY5Y Human Neuroblastoma Cells Exposed to Insulin, IGF-1, and Muscarinic Agonists

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Laser-captured hippocampal dentate granule neurons from two separate cohorts of normal controls and schizophrenics were examined and compared with bipolar disease and major depressive disorder cases. The microarray studies revealed in both schizophrenia cohorts, but neither in depression nor bipolar disease, decreases in large clusters of genes that encode for energy metabolism and mitochondrial oxidative functions, including isocitrate, lactate, malate, NADH and succinate dehydrogenases; cytochrome C oxidase and ATP synthase. Decreases in genes encoding for brain mitochondria, energy metabolism, ATP production, and hydrogen ion transport in hypofunctioning hippocampal neurons may contribute to the cognitive and sensory processing deficits of schizophrenia. Interestingly, decreases in many of these same genes have been reported in microarray studies of human and rodent diabetic skeletal muscle, and insulin exposure in vivo can reverse these decreases. To evaluate insulin responosivity in a model cell system, the human SH-SY5Y neuroblastoma cell line and human astrocytes were exposed to insulin or insulin-like growth factor-1 (IGF-1) to determine if increases in insulin signaling could reverse the pattern of gene changes characteristic of schizophrenia. Insulin induced a phosphorylation of insulin receptors in the SH-SY5Y cells but not the astrocytes and, like IGF-1, increased phosphorylation of the insulin-signaling mediators ERK1/2 and Akt. The ERK inhibitor PD98059 blocked ERK1/2 phosphorylation by either hormone. Insulin and IGF-1 but not BDNF increased the expression of many genes that overlapped with genes decreased in the hippocampus of multiple schizophrenic cohorts, including those involved in mitochondrial functions, glucose and energy metabolism, and hydrogen ion transport. Representative genes from each of these classes were incorporated into a 16 gene, oligonucleotide-based miniarray for compound screening. Unlike IGF-2, bFGF or EGF, Insulin and IGF-1 again produced dose-related alterations in gene expression identical to those determined with microarrays. While most of 2,400 pharmacologically defined compounds failed to alter gene expression, muscarinic agonists mimicked the insulin gene profile. The muscarinic antagonist atropine and M1-preferring antagonist telenzepine blocked these effects. Increases in insulin/IGF-1 or muscarinic signaling may normalize genomic alterations in schizophrenia and thereby lead to treatments that better address the root causes of this disease. We will discuss the relevance of insulin and muscarinic agonism to clinical improvements seen in psychoses, and implications of muscarinic antagonism to a worsening of cognitive functions in schizophrenia, and to the diabetogenic effect observed with typical and atypical antipsychotic agents.

Insulin Receptor Deficits in Schizophrenia and in Cellular and Animal Models of Insulin Receptor Dysfunction

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Schizophrenia is associated with abnormalities in glucose metabolism that may lead to insulin resistance and the observed increase in the incidence of Type II diabetes mellitus. The role(s) of insulin-dependent Akt signaling, animal and cellular models of insulin resistance, and mitochondrial enzymes of energy metabolism were studied in schizophrenia. Postmortem studies revealed a functional decrease in insulin receptor (IR)-mediated signal transduction in the dorsolateral prefrontal cortex of persons with schizophrenia vs. controls. Content and auto-phosphorylation levels of IRβ were reduced by approximately 50% and Akt content and activity (pSer473-Akt) were decreased by ~76-78%. The inhibition of IRβ signaling was accompanied by elevated contents of glycogen synthase kinase (GSK)-3α and GSK-3b without significant changes in phospho-Ser21/9 GSK-3α/β levels. As in schizophrenia, IRb knockdown in an siRNA cellular model demonstrated a reduction in the Akt content and activity. Total GSK-3α/β content remained unaltered, but phospho-Ser21/9 GSK-3α/β levels were reduced causing a net increase in the overall enzyme activity similar to that in schizophrenia. Insulin resistance was induced in mice by treatment with clozapine. Behavioral testing showed decreases in startle response magnitude in animals treated with clozapine for 68 days. Clozapine treatment resulted in a functional inhibition of IRβ but Akt activation status remained unaltered. In contrast to schizophrenia, changes in GSK-3α/β in clozapine treated mice were consistent with a net decrease in enzyme activity. These results suggest that alterations in insulin-dependent Akt signaling in schizophrenia are similar to those observed in the cellular but not clozapine treatment-dependent animal models of insulin resistance. That insulin resistance can lead to cognitive (spatial learning and memory) impairments was confirmed in an obesity model of insulin resistance in mice. In separate experiments the activities of energy metabolism enzymes of the of the Krebs tricarboxylic acid cycle were studied. Activities of aconitase, α-ketoglutarate dehydrogenase complex and succinate thiokinase decreased significantly, whereas those of succinate dehydrogenase and malate dehydrogenase increased providing additional evidence for functional abnormalities in components of oxidative/glucose metabolism pathways. Taken together these studies suggest that aberrant IR function and Krebs cycle enzymes may be important in the pathophysiology of schizophrenia.

Insulin Regulation of Molecular Signaling Pathways Responsible For Associative Memory

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Background: An accumulating body of evidence has suggested that insulin's role in brain function is not limited to the regulation of glucose metabolism in and/or uptake by brain cells. Rather, via insulin receptors and insulin-like growth factor receptors (IGF receptors), insulin can also modulate synaptic function and/or modifiability

through effects on glutamatergic and GABA synaptic receptors. In addition, via the IR and IGF receptors, insulin can regulate signaling pathways that are responsible for associative learning and memory. Such pathways include 1) the IR-Shc-MAP kinase pathway that also regulates gene expression, and 2) the G-protein-coupled receptor PLC pathway that leads to PKC isozyme activation and the sequence of downstream protein substrates that control and modify synaptic efficacy.
Methods: Subcellular synaptic membrane and cytosolic fractions were analyzed with Western blots, immunohistochemistry, immunoprecipitation, phosphorylation assays, in situ hybridization, and immuno-binding studies for Shc, IR, IGF, non-receptor Src tyrosine kinase, MAP kinase Erk1/2 and other molecular steps in insulin-PKC signaling cascades during rat spatial maze training and control paradigms.
Results: Rat maze training up-regulated IR mRNA in the CA2 and dentate gyrus of the hippocampus and increased levels of hippocampal IR protein. In the hippocampal CA1 pyramidal neurons, changes in the distribution pattern of IR in specific somatic and dendritic compartments were found only in maze-trained animals (Zhao et al., J. Biol. Chem., 1999). Although IR showed a low level of in vivo tyrosine phosphorylation, an insulin-stimulated increase of in vitro Tyr phosphorylation of IR was found only in trained animals. Furthermore, a training-induced co-immunoprecipitation of IR with Shc-66 occurred in trained animals together with in vivo Tyr phosphorylation of Shc and MAP kinase as well as accumulation of Shc-66, Shc-52, and Grb-2 in hippocampal synaptic membrane fractions. The IR also interacts with the non-receptor tyrosine kinase, pp60 c-src, in rat hippocampal synaptic membrane fractions. Training in the water maze caused enhanced interactions of c-src with synaptic proteins such as synapsin 1, synaptophysin, the NMDA receptor, and cytoskeletal protein, actin, but transiently reduced binding to the IR.
Conclusion: These and a variety of other observations implicate insulin signaling pathways in the formation of associative memory and may help explain how the deterioration of insulin receptor signaling could contribute to age-related cognitive impairment and dementias such as Alzheimer's disease.

Panel Session
Pharmacology and Molecular Genetics of Nicotinic ACh Receptors: Relevance for Treating Nicotine Addiction

Nicotinic Receptors: Modulation by Nicotine

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Background: Nicotinic acetylcholine receptors (nAChRs)are the proximal site of action of nicotine from tobacco. Recent evidence demonstrates that there are multiple nAChRs, each with unique regional distributions and proposed functions. Furthermore, nAChRs numbers and functions change with chronic exposure to nicotine. In order to understand the role of nicotine in smoking, as well as the actions of therapeutic agents for smoking cessation, it is necessary to understand nAChR subtypes, their regional distributions, their proposed functions, and how these receptors adapt to chronic nicotine administration and how they can be exploited as targets for treating nicotine addiction.
Methods: A general overview of nAChRs from receptor binding, to regional anatomical localization, to behavioral pharmacology will be the methods reviewed for a full and comprehensive background on nAChRs. A review of systems neuroscience and the complex pharmacology of nAChRs provide a working hypothesis for how nicotine itself modifies both nAChRs and dopamine release to produce the consequences of smoking, including subjective reinforcing effects, dependence, craving and withdrawal. Modulating the ability of nicotine to interact with all subtypes of nAChRs, but with a different pattern of receptor occupancy due to chronic delivery of the nicotine agonist, is the principle behind nicotine replacement therapies. Preventing nicotine from reaching nAChRs with a nicotine "vaccine" is another approach. Targeting the specific nAChRs thought to mediate the reinforcing and dependence of nicotine is yet another approach. All these actions will be reviewed here.
Results: Nicotine produces a myriad of pharmacodynamic actions in the body through interactions with multiple subtypes of nAChRs. Various nAChRs are expressed in a regionally selective manner that would enable nicotine to influence neural circuits associated with autonomic, hormonal, emotional, and executive function processes. Critically, nicotine addiction results from nAChR activation at neural systems (e.g.,dopamine) that support nicotine's reinforcing effects. In fact one treatment for smoking cessation does not target nicotinic receptors directly, but rather targets the theoretical consequences of nicotine action on dopaminergic reward neurons, namely the antidepressant bupropion, which modulates dopamine and noradrenergic pathways that influence neural circuits involved in reward/ attention/ craving/ withdrawal downstream to the effects of nicotine. Administration of nicotine itself also has profound actions over time on the regulation of nAChRs which in turn influences calcineurin / CREB –dependent signaling pathways. A novel approach that has been gaining scientific momentum is partial agonism of nAChRs to competitively attenuate, but not completely block,the downstream responses of nicotine. Theoretically, such an approach would require sufficient agonist actions to mitigate the craving of nicotine withdrawal yet sufficient interference with nicotine actions to block the effects of exogenous nicotine.
Conclusion: Understanding the specific nicotinic receptor subtypes involved in mediating the reinforcing actions of nicotine, the site of these receptors, and how they become dysregulated with nicotine dependence is key to understanding how to target these receptors in novel ways for more robust therapeutic treatments of nicotine addiction. Nicotine has profound and relatively fast onset of actions on nAChRs that accompany reinforcement, dependence and withdrawal from this agent. Results provide converging support that one of the key subytpes of nAChRs involved in nicotine addiction is the alpha4beta2 subtype. Interfering with nicotine's action at this receptor subtype may also represent a novel approach to a next generation smoking cessation therapy.

Nicotinic Acetylcholine Receptors and Signaling Pathways Involved in Behaviors Related to Nicotine Addiction

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Background: Knockout mice lacking the nAChR beta 2 subunit show impaired performance in behavioral paradigms related to nicotine addiction. Beta 2 subunit-containing nAChRs are present throughout the brain in many of the regions and circuits that mediate these behaviors. In addition, nicotine administration to experimental animals leads to behavioral plasticity such as locomotor sensitization and place preference, as well as changes in the expression and activation state of a number of signal transduction molecules. Previous work in our laboratory has identified intracellular signaling pathways activated by nicotine, including pathways involving calcineurin (protein phosphatase 2B) and CREB.
Methods: We have generated several lines of transgenic mice that express the nAChR beta 2 subunit inducibly in different brain areas and subsets of neurons including dopaminergic neurons, corticothalamic effects and visual relay nuclei. We have tested transgenic mice in behavioral paradigms related to nicotine addiction including locomotor sensitization and place preference. We have also determined whether modulation of intracellular signaling pathways downstream of nAChR activation in wild type rodents affects these behaviors using pharmacological and viral vector based strategies.
Results: Testing lines of mice with differing patterns of expression has allowed us to anatomically and pharmacologically dissect the actions of nicotine in the brain. For example, nicotine-induced locomotor activation is rescued in mice with local expression of beta 2 nAChRs in dopamine neurons. By contrast, systemic or VTA blockade of cal-

cineurin attenuates nicotine-dependent locomotor sensitization. Similarly, global knockout of beta 2 nAChRs abolishes nicotine place preference, and manipulation of either calcineurin activity pharmacologically, or CREB activity with a viral vector expressing a dominant negative CREB in the nucleus accumbens also demonstrates an important role for these signaling molecules in nicotine place preference. **Conclusion:** Our results thus far identify beta 2 nAChRs on dopamine neurons as critical for locomotor activation by nicotine and suggest that both calcineurin and CREB influence several aspects of nicotine-induced behavior. These molecules appear to be important modulators of nicotine-induced plasticity.

Nicotine Addiction Begins with the First Cigarette

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Background: Recent reports establish that withdrawal symptoms are common among adolescents after a few weeks of intermittent tobacco use. No established model of nicotine dependence predicted the development of symptoms of dependence and withdrawal prior to the development of tolerance.
Methods: The neuroscience literature was combined with clinical observations to develop the sensitization-homeostasis model of how nicotine dependence develops, intensifies and resolves in humans. Several parts of this model have been confirmed experimentally.
Results: In a nutshell, nicotine causes addiction by stimulating brain areas that are responsible for the suppression of craving. The process of sensitization amplifies the craving suppression produced by nicotine to super-physiologic levels. The super-inhibition of the craving neurons evokes compensatory homeostatic measures. When nicotine is absent, these compensatory adaptations autonomously stimulate craving. The processes that lead to nicotine dependence begin with the first cigarette and are well established before the onset of daily smoking.
Conclusion: The model will be used to explain how medications can help in the treatment of nicotine addiction.

Pharmacology of Varenicline, a Clinically Effective Alpha4beta2 Nicotinic Acetylcholine Receptor Partial Agonist for Smoking Cessation

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Background: We have pursued smoking cessation therapy with a partial agonist of alpha4beta2 nicotinic acetylcholine receptors (nAChRs), which mediate the reinforcing and dependence-associated effects of nicotine in the mesolimbic pathway, as a novel approach to aid in smoking cessation. A partial agonist can act as an agonist and relieve craving and withdrawal symptoms in smokers who quit, and as an antagonist,can reduce the reinforcing effects of tobacco use.
Methods: Compounds with high in vitro binding affinity and partial agonist functional efficacy at alpha4beta2 nAChRs were selected for in vivo testing. Compounds that had partial agonist effects on mesolimbic dopamine release in vivo were subsequently studied in animal models of nicotine reinforcement.
Results: Varenicline was identified as an alpha4beta2 nAChR partial agonist with the desired subnanomolar affinity and ~50% of nicotine's maximal efficacy at alpha4beta2 nAChR receptors. In vivo, varenicline is significantly less efficacious, but more potent, than nicotine in stimulating dopamine release in rat nucleus accumbens and effectively attenuates nicotine-induced dopamine release. Varenicline reduced nicotine self-administration and supported lower self-administration break points than nicotine.
Conclusion: Varenicline's neurochemical and pharmacological profile reflects potent and selective alpha4beta2 nAChR partial agonist activity with lower intrinsic efficacy than nicotine. It is orally active in reducing nicotine self-administration, an animal model for smoking behavior. These data suggest that varenicline could partially reproduce the subjective effects of smoking by (moderately) activating alpha4beta2 nAChRs, while preventing nicotine to fully activate alpha4beta2 nAChRs. Varenicline displays excellent pharmacokinetic properties and is in clinical development as a therapeutic aid for smoking cessation. Results of these studies demonstrate that varenicline can significantly improve quit rates compared to placebo. With its novel pharmacology it has the potential to provide a more effective therapy for smoking cessation than those currently available.

Panel Session
Psychiatric Diagnosis Revisited in the Era of Molecular Genetics

The Genetics of Pschosis: Breaking Down Diagnostic Boundaries

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A number of lines of evidence suggest that schizophrenia and bipolar disorder share overlapping etiological and pathological processes. Evidence from genetic epidemiology has traditionally been interpreted to support a dichotomous view of two discrete disorders, although recently emerging, as well as classical data do not fit well with this model. Moreover, the pattern of findings emerging from molecular genetics shows increasing evidence for an overlap of genetic susceptibility across traditional calssification categories. This includes association findings at DAOA(G72), DTNBP1 (dysbindin), COMT, BDNF, DISC1, and NRG1. These will be discussed and the emerging evidence suggests the possibility of relatively specific relationships between genotype and psychopathology. For example, DISC1 and NRG1 may confer susceptibility to a form of illness with mixed schizophrenic and affective features, whereas DAOA appears to confer risk to bipolar disorder and only in cases of schizophrenia where episodes of mood disoder also have occurred. The elucidation of genotype - phenotype relationships is at an early stage, but current findings highlight the need to consider alternative approaches to classification and conceptualization for psychaitric research rather than continuing to rely heavily on the traditional Kraepelinian dichotomy.

Psychiatric Diagnosis: Tracking Genes and Symptoms Through Disease Catagories

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Kraeplin's categorization of major psychosis into dementia preaecox and manic depressive insanity has been accepted in modern psychiatric classification schemes. However according to current classification systems, the establishment of a psychaitric diagnosis remains dependent solely on the presence of characteristic symptoms and their duration. In view of the large overlap of symptoms between psychiatric disorders, particularly schizophrenia and bipolar disorder, the validity of these systems has been repeatedly re-examined and hotly debated. This debate is reflected in the existence of several classification systems including the DSM, ICD, RDC, Leonardian and Schneiderian criteria. Even the standard American and European systems the DSM and ICD are subject to continuous revision. Furthermore psychaitric disorders demonstrate a high degree of co-morbidity and the degree to which these co-morbid disorders actually belong to the original disorder is unclear. The molecular genetic approach allows not only the association of genetic variants with specific disorders but also symptoms which have been shown to be heritable and their underlying biological traits. This approach is examplified by the sssociation findings with the G72/G30 gene. We found an association with schizophrenia (SZ) as well as bipolar disorder (BPD) in two German

samples. Furthermore, it was shown that the association between BPD and the G72/G30 varient is largely accounted for by those BPD patients with a history of persecutory delusions and in SZ with patients having the paranoid subtype. Given that persecutory delusions are characterizby the presence of fear, anxiety related traits may be at the core of the association between BPD, SZ and the G72/G30 locus. It is therefore intriguing that we also found evidence for an association between Panic Disorder, a prime example of an anxiety disorder and the same genotypes and hapolotypes at the G72/G30 locus. Ongoing analysis in a preliminary sample of patients with depression show the same association. The genotyping of larger samples of with and without anxiety is underway. These findings demonstrate that certain genes cross diagnostic boundaries and may be responsible for specific symptoms and biological mechaisms underlying those symptoms in a variety of illnesses.

Genetically Determined Psychopathology Across Diagnostic Categories: Evidence and Implications

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Genetically determined pathophysiology has most clearly been demonstrated in cases of single gene mutations such as seen in Huntington's Disease. Here changes in the number of triplet repeats in one gene determine if the illness develops and the age of onset. In this case the progression of the disorder results in a variety of symptoms which can resemble mania at one point in time and paranoid schizophrenia at another. The bewildering flux in psychopathological symptoms appears to be related to the progression of the degenerative process and not to the alterations in the specific gene responsible. In the case of familial Alzheimer's Disease we know of over 50 mutations on 3 genes that will lead to similar forms of psychopathology with different ages of onset and rates of progression. This is explained by the fact that all the mutations result in a change in the processing of APP. Thus a common biochemical alteration results in the pathophysiology. In the case of the major psychosis it appears that no single gene accounts for a major proportion of the varience but that a variety of genes may play a role in several disorders. It also appears that these genes may be associated with specific psychopathological features. In the case of G72/G30 we have shown a possible relationship between a specific haplotype and irrational fear. The question is can one find evidence that a common pathophysiology in association with this haplotype. As a first attempt we examined bipolar patients with paranoia and paranoid schizophrenic patients using structural MRI in order to see if there where common morphological changes. We found changes in the superior temporal lobe and DLPFC in both groups with persecutory delusions and no such changes in bipolar patients without persecutory delusions. There appears to be a thinning of the cortex in the superior temporal lobe which may be associated with a specific haplotype of the G72/G30 gene. This suggests that a specific form of cortical pathophysiology may be associated with this haplotype and the symptoms associated with it. This has implications for therapy as well as diagnostic classification, which will be discussed.

Biological and Psychosocial Precepts in Developmental Outcome: The Interplay of Genetics, Epigenesis and Environmental Cues

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Background: Dynamic interactions of genes and environment during the perinatal period lead to '"programming" of physiological and behavioral response systems giving rise to individual differences that may be either adaptive or pathological. This process operates within an envelope specified by the organism's genome and further delineated by the presence or absence of particular alleles. Information concerning environmental stability vs. instability, availability of nutritional sources, and other stressors during fetal development and early childhood must first be detected and encoded, probably via epigenetic modifications. Overall, this information acts as a basis for matching the development of stress response systems such as the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system, and neurocircuits involved in emotion regulation to "predicted" demand.

Methods: Studies have been performed on rodents involving: (1) exposure to perinatal stressors, (2) use of animals +/-1.5 SD from the central mean on a variety of measures, (3) retrovirally-mediated gene transfer of a corticotropin releasing factor (CRF) over-expression construct in the neonatal period to mimic observations from prior experiments, (4) determining the presence of polymorphisms, creating "humanized" transgenic lines, and mapping chromatin modifications.

Results: Our data support early modifications of developing CRF and aminergic (5-HT, NE, DA) neurocircuits in response to perinatal stressor exposure in the form of a prenatal immune challenge, prenatal maternal restraint, or neonatal maternal separation. Between 30-40% of the offspring can be characterized as exhibiting high anxiety-like behavior, displaying a hypohedonic state, having a distinct ethanol preference, and showing HPA axis hyper-responsiveness. An increased incidence of elevated blood pressure and metabolic disease is evident in these animals at 8-12 mo of age. However, not all rats necessarily display every aspect of this complex phenotype. The individual variability in outcome may be related to the presence or absence of polymorphic genes along the CRF and/or aminergic networks or along impinging regulatory networks such as brain derived neurotrophic factor (BDNF) or in elements of intracellular signaling cascades.

Conclusion: The animal models described yield reasonably parallel outcomes and point toward the action of similar mechanisms. We propose that perinatal stressor exposure occurring during sensitive periods along the normal developmental trajectory act to "fine tune" the responsiveness of major physiological and behavioral regulatory systems. The influence of genes and environment are integrated into a "response threshold" that modulates the overall regulatory cascade leading toward one of numerous phenotypic pathways. The overall effect of a stressor is dependent upon the genetic constitution of the parents and offspring, avidity of epigenetic mechanisms, the developmental timing of stressor exposure, stressor magnitude and duration, and whether exposure represented a "one-shot" or rare event vs. repeated or chronic exposure. In such a model, exposure to environmental adversity (or stability) activates a developmentally sensitive regulatory cascade that, through epigenetic mechanisms, serves to program stress-responsive and other systems toward hypo- or hyper-responsivity in tune with putative environmental demands. The influences of genes and environment are integrated into a "response threshold" that then modulates the overall regulatory cascade.

Panel Session
Treating Affective Disorders - Moving from Resetting Chemical Imbalance to Targeted Neuromodulatory Interventions

Corticolimbic Gray and White Matter Targets for the Treatment of Bipolar Disorder

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Background: Convergent evidence supports the presence of gray and white matter abnormalities in corticolimbic neural circuits that subserve adaptive regulation of emotions and motivation in bipolar disorder (BD). Increasing evidence implicates abnormalities in the white matter connections between these brain regions, and suggests the importance of functional interactions between the regions in BD

pathology and its treatment. We sought to study the relationship between gray and white corticolimbic abnormalities in BD and to consider new targets for its treatment.
Methods: Subjects with and without BD participated in multimodality neuroimaging study that included structural magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI) and diffusion tensor MRI (DT-MRI) scanning. Analyses of corticolimbic regional structure, function and connectivity were performed. Influences of mood state and specific pharmacotherapies were explored.
Results: The neuroimaging findings support the involvement of regional structural and functional disturbances in mesial temporal, ventral striatal and ventral anterior cingulate regions in BD. The data are consistent with theories that reversal of abnormalities in these brain regions may contribute to the salutary effects of mood-stabilizing pharmacotherapies. New data also support the presence of prominent white matter connectivity abnormalities in BD. For example, abnormalities were detected in the anterior cingulum, a major white matter bundle that provides substantial connectivity within the implicated corticolimbic circuitry and that is proximal to current neuroanatomically targeted interventions.
Discussion: The findings provide further support for the anatomically-specific treatments to be discussed in this panel. The new structural and functional connectivity analyses raise questions about novel, potential treatment targets such as in corticolimbic white matter.

Preclinical Studies of Affective and Anxiety-Related Brain Circuitry in Nonhuman Primates
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Background: Studies in nonhuman primates examining the neural circuitry underlying affective processing provide an ideal translational linkage between mechanistic studies in rodents and clinical reseach. Furthermore, monkeys that display patholgoical emotional responses can be readily identified and studying them allows for an uderstanding of mechanisms involved in mediating human psychopathology.
Methods: In addition to studying fear and anxiety-related behaviors, FDG PET studies were performed in rhesus monkeys to understand patterns of brain metabolic activity that are associated with adaptive and maladaptive responses. In addition, selective lesioning strategies were employed to examine the mechanistic role of the amygdala and prefrontal cortical regions in mediating anxiety related behavioral and physiological alterations.
Results: Functional brain imaging studies reveal an association between metabolic activity in limbic structures such as the bed nucleus of the stria terminalis and the amygdala with threat-induced behavioral inibition. Lesions of the central nucleus of the amygdala or the orbitofrontal cortex decreased behavioral inhibition and snake fear. Central nucleus lesions also decreased cortisol and CSF CRF concentrations. The orbitofrontal lesions were without effect on these parameters but altered patterns of frontal brain electrical activity.
Discussion: Taken together, these results provide evidence for an affective neural circuit in primates that is involved in the regulation of affective and anxiety related behavior and physiological responses. Along with other data, these findings provide support for interventions in humans that might be targeted at specific brain sites.

Putative Targets for Neuromodulation Identified by Functional Neuroimaging
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Background: Functional neuroimaging has provided an important platform to define critical brain circuits involved in both depression pathogenesis and treatment response. Such strategies are the foundation for development and testing of potential novel therapeutic interventions using neuromodulation.
Methods: Converging findings from a series of positron emission tomography studies examining brain changes mediating depression remission to pharmacological and non-pharmacological antidepressant interventions were examined using a combination of (1) linear pre-post treatment comparisons, and (2) multivariate modeling of baseline scan patterns predictive of specific treatment outcomes. Similarities and differences among interventions were interpreted in the context of a pre-defined network depression model.
Results: Subdivisions of the anterior cingulate cortex were repeatedly identified using this depression network approach. Most consistent were alternations in patterns of subgenual cingulate (Cg25) connectivity to specific cortical and subcortical targets. Cg25 connections to the brainstem, hypothalamus, and insula have been linked to disturbances of circadian regulation (sleep, appetite, libido, neuroendocrine changes), whereas reciprocal pathways between Cg25 and orbitofrontal, medial prefrontal, and more dorsal aspects of the anterior and posterior cingulate cortices form the neuroanatomical substrates by which primary autonomic and homeostatic processes might influence various aspects of learning, memory, motivation and reward – all core behaviors altered in depressed patients. Differential metabolic effects on these network subsystems by pharmacotherapy and psychotherapy were consistent with putative mechanisms mediating these various interventions.
Conclusion: Systematic studies of regional brain changes mediating antidepressant response across diverse treatments have identified an important point of intersection at the level of the subgenual cingulate (Cg25). These findings have subsequently served as the basis for development and testing of a novel new treatment strategy using chronic, high frequency brain stimulation (DBS) to selectively modulate dysfunctional subgenual cingulate connectivity in treatment resistant depressed patients. Clinical response in patients receiving this treatment and correlative functional neuroimaging studies mapping the effects of chronic Cg25 DBS support the critical role of this region in major depression and its treatment.

Targeted Neuromodulation in Affective Disorders – Hypotheses, First Results and Outlook
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Psychotropic drugs work by altering neurochemistry to a large extent in widespread regions of the brain, many of which may be unrelated to depression. Deep Brain Stimulation (DBS) allows the neuromodulation of sites in the brain implicated in major depression. Because the most prominent clinical feature of depression is anhedonia — the inability to experience pleasure from previously pleasurable activities — and because there is clear evidence of dysfunctions of the reward system in depression, DBS to the Nucleus Accumbens might offer a new possibility to target depressive symptomatology in otherwise treatment-resistant depression. Six patients suffering from extremely refractory forms of depression, who did not respond to pharmacotherapy, psychotherapy and electroconvulsive therapy, were implanted with bilateral DBS electrodes in the nucleus accumbens. Stimulation parameters were modified in a double-blind fashion, and clinical ratings were assessed at each modification. In addition, brain metabolism was assessed one week before and one week after the onset of stimulation. Clinical ratings improved significantly in all patients when the stimulator was on, and worsened in all three patients when the stimulator was turned off. Effects were immediate, and no side effects occurred in any of the patients. Using FDG-PET, significant changes in brain metabolism as a function of the stimulation in frontostriatal networks were observed. Dysfunctions of the reward system – in which the nucleus accumbens is a key structure – are implicated in the neurobiology of major depression and might be responsible for impaired reward processing, as evidenced by the symptom of anhedonia. Today it cannot be assumed that DBS will cure treatment refractory depression.

Clinical usefulness of DBS approaches at different target sites still needs to be demonstrated convincingly. Several ethic al issues specific to neurosurgical methods in refractory psychiatric patients will have to be dealt with in parallel to rigorous scientific assessment of antidepressant efficacy. Hypothesis guided Deep Brain Stimulation of different targets may reveal more information on the underlying neurobiology of depression and could be an interesting putative new antidepressant approach.

Panel Session
Bipolar Disorder: What is the Core Deficit?

Core Cognitive Deficits in Bipolar Disorder

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Cognitive impairment is increasingly recognized as an important feature of bipolar disorder and is associated with poor functional outcome. For example, the cognitive difficulties of these patients contribute to lack of organization and planning, suboptimal decision making, poor compliance with treatment and limited ability to engage in psychological treatments and rehabilitation. This talk will focus on recent data examining neurocognition in the manic, depressed and euthymic states to determine the core cognitive deficits in bipolar disorder. In addition, novel neuropsychological and neuroimaging data comparing unipolar and bipolar depression will be presented (Taylor Tavares, Clark, Williams, Furey, Sahakian and Drevets, unpublished). These data will provide objective measures for assessing the efficacy of novel treatments and for identifying vulnerable individuals in at risk populations. Finally, the importance of the concept of 'cognitive reserve' as applied to bipolar and other neuropsychiatric disorders will be discussed. Acknowledgement - This work was funded in part by the Wellcome Trust, Medical Research Council and National Institutes of Health (NIMH). Declaration of Interest - Consultant for Cambridge Cognition Ltd.

Increased Amygdala Activity Toward Positive but not Negative Emotional Stimuli: A Potential Biomarker of Bipolar Disorder

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Background: We have aimed to identify potential biomarkers of bipolar disorder by examining persistent and bipolar-specific abnormalities in performance of emotion processing tasks, and in neural systems underlying emotion and cognition.
Methods: We measured performance accuracy and response bias in labeling neutral, mild and intense happy and sad facial expressions from standardized series in remitted and depressed adults with bipolar disorder type I (BPI), depressed adults with unipolar depression, and age-matched healthy individuals. Using functional neuroimaging, we measured neural activity to positive and negative emotional stimuli in remitted and depressed young adults with BPI, remitted and depressed adults with unipolar depression, and age-matched healthy individuals. Here, we employed two experimental paradigms: 1. displays of standardized facial expressions of happiness, sadness and fear during gender labeling; and 2. presentation of emotional words embedded in a working memory task (digit sorting).
Results: On the facial expression labeling task, remitted individuals with BPI did not show any expression labeling abnormalities. Subtle biases toward labeling mild intensity sad expressions as happy were demonstrated by depressed individuals with BPI. In contrast, unipolar depressed individuals showed a bias toward labeling mild intensity happy expressions as neutral. Compared with healthy individuals both remitted and depressed individuals with BPI showed increased amygdala activity to happy, but not fear or sad, facial expressions. Both remitted and depressed individuals with BPI also showed increased and sustained amygdala activity after positive words during subsequent digit sorting. This pattern of abnormal amygdala activity during both experimental paradigms was not shown by unipolar depressed individuals. Depressed individuals with BPI also showed relative increases in dorsolateral prefrontal cortical activity during digit sorting after presentation of positive, but not negative or neutral, words. No between-group differences were observed in amygdala and dorsal prefrontal cortical volumes.
Conclusions: These findings suggest subtle biases in emotion labeling distinguishing depressed individuals with BPI from unipolar depressed individuals. Our data from neuroimaging studies indicate that patterns of abnormally increased amygdala and prefrontal cortical activity to positive but not negative emotional stimuli are specific to BPI and not present in individuals with unipolar depression. Furthermore, our data suggest that the pattern of abnormally increased amygdala activity to positive but not negative emotional stimuli persists during depression and remission in BPI, and may thus represent a biomarker of the disorder. This pattern of abnormally increased amygdala activity to positive emotional stimuli may underlie the predisposition to mania in individuals with BPI, and is a potential focus for future studies examining biomarkers of the disorder.

Amygdala Deficits in Bipolar Disorder vs. Anxiety Disorders: It's All About Context

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Arguably, the amygdala has been the focus of more psychiatric research than any other brain region. Amygdala dysfunction has been implicated in bipolar disorder(BD), major depression, and anxiety disorders(ANX). Is the amygdala dysfunctional in all of these illnesses and, if so, how does the deficit in one disorder differ from that in another? The interface between anxiety and BD is important because longitudinal data indicate that children with ANX are at increased risk for BD in early adulthood. Also,comorbid ANX is associated with increased morbidity in BD, and there may be genetic associations between ANX and BD. By comparing patients with BD and comorbid anxiety disorders (BD+ANX), those with BD but no anxiety disorder (BD-ANX), and those with anxiety disorders alone (ANX), researchers can dissociate the neural deficits of BD from those of ANX and make progress toward identifying deficits unique to BD. We present unpublished behavioral and fMRI data comparing children with BD-ANX, BD+ANX, and ANX; the fMRI data provide insight as to how amygdala dysfunction in BD may differ from that in ANX. We first studied the impact of comorbid anxiety on the pathophysiology of BD with a dot probe task that has been used to identify cognitive biases in ANX. In an unpublished behavioral study, we found that BD+ANX (N=20) had an attentional bias toward threatening faces. This bias differentiated BD+ANX from BD-ANX (N=11); the latter did not differ from controls (N=14). Published data indicate that children with ANX alone have an attentional bias away from threatening faces, i.e. opposite in direction to what we found in BD+ANX. These data identify neurocognitive differences between BD+ANX and BD-ANX, as well as between BD+ANX and ANX; they also indicate that ANX and BD have dissociable effects on attentional function in the presence of emotional stimuli. We then conducted an fMRI study to follow up on these data. In particular, we were interested in ascertaining how amygdala function might vary among BD+ANX (N=14), BD-ANX (N=19), ANX (N=22), and controls (N=24). In this unpublished study, subjects viewed faces (neutral, fearful, angry, happy) while their attention was directed toward their subjective response to the face, the emotion on the face, or a nonemotional facial feature. Behaviorally, BD patients rated themselves as more fearful than either ANX or controls when viewing neutral faces. fMRI data indicated a double dissociation in amygdala dysfunction. Specifically, compared to ANX or controls, the sample that included all BD patients had amygdala hyperactivation when viewing neutral faces and rating their fear vs. the nonemotional condition ("How afraid are you?" vs. "How wide is the nose?"). The same abnormality was present

when only BD+ANX were compared to ANX. On the other hand, ANX, compared to BD or controls, had amygdala hyperactivation on the same "how afraid?" vs. "nose width?" contrast, but when viewing fearful, rather than neutral, faces. These data suggest that BD patients differ from both ANX and controls in that they react to nonemotional, neutral stimuli as if they were emotional; in patients with BD, amygdala activation occurs in contexts that are viewed by others as neutral. In ANX, abnormal amygdala activation is also present, but here it occurs when viewing a stimulus associated with the presence of threat (i.e. a fearful face). Of note, in order to identify this between-group difference in amygdala dysfunction, it was essential to use a paradigm that manipulated attention experimentally and to acquire behavioral data during scanning.

Signaling Cascades Regulate Glutamatergically-Mediated Neural Plasticity in Critical Limbic and Reward Circuits: Implications for the Pathophysiology and Treatment of Bipolar Disorder

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Objectives: To test the hypothesis that the core cellular defect in BD is in the ability to regulate neuroplastic adaptations to perturbations (both physiological and pathophysiological)—an inability to handle "normal loads" (neurochemical, hormonal, stress-induced, pharmacologically induced, etc.) without failing, or invoking compensatory adaptations that overshoot and predispose to oscillations. Many of the very same "plasticity regulators" also play a critical role in cell survival, cell death, and cellular resilience, thereby serving to explain the atrophic (perhaps degenerative) aspect of the illness in some patients, as well as the presence of stigmata normally associated with ischemic/hypoxic insults, such as white matter hyperintensities.
Methods: The BDNF/extracellular signal regulated kinase (ERK) and GSK-3 pathways are signaling cascades conveying the actions of neurotrophins, neurotransmitters and neuromodulators on cellular plasticity and resilience. We – and others – have shown that these pathways are major targets of antidepressants and mood stabilizers, and have thus been postulated to play important roles in the tx of BD. We have therefore undertaken a complimentary series of molecular, cellular and behavioral studies to elucidate the role of signaling cascades regulate glutamatergically-mediated neural and behavioral plasticity in critical limbic and reward circuits.
Results: In a series of studies, using knockout and transgenic animals as well as lentiviral siRNA and TAT-peptide (an HIV-coat protein which assists in the delivery of target peptides across the BBB and into neurons) administration, we have found that perturbing these pathways exerts major effects on behaviors which recapitulate much of the core behavioral sx of BD – including responses to psychostimulants, motoric behavior, risk taking behavior and hedonic drive. This was observed either with either genetically modified animals, chemical signal transduction modifiers, or lentiviral dominant negative strategies, providing strong confirmatory evidence. Additional cellular studies have demonstrated that the intracellular cascades are translated into behavioral changes by regulating glutamatergically-mediated synaptic plasticity. Notably, they regulate the trafficking and synaptic insertion of AMPA GluR1 and GluR2 receptors in limbic circuits; furthermore the causal relationship with behavioral plasticity has been delineated by the use of TAT-peptides to regulate AMPA trafficking. While the precise susceptibility genes for BD have not yet fully been elucidated, emerging data implicates GRIK2 (which codes for the GluR6 receptor). Our new studies also show that genetic manipulation of GluR6 receptors exerts major effects on persistent hyperactivity, escalated irritability and aggression, risk taking, and hyperhedonia – all critical facets of the manic syndrome.
Conclusions: The data supports that hypothesis that the core defects in BD may arise from impairments in cellular plasticity cascades in critical limbic synapses and circuits. These defects offer considerably explanatory power to explain not only the core behavioral features of BD, but also other facets of the illness including antidepressant-induced switches, cycle acceleration and the atrophic (sometimes progressive) changes observed in some patients. The identification of glutmatergically mediated synaptic plasticity as a target for cellular plasticity cascades implicated in the pathophysiology and tx of BD holds the potential to lead to the development of improved Rx for this devastating illness.

Panel Session
Large Scale Pharmacogenetics in Large-Scale Trials

The Genetic Basis of Treatment Response in Schizophrenia

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Background: Although there has been substantial progress in the pharmacotherapy of schizophrenia over the past half century, numerous limitations of treatment persist and many critically important clinical questions remain wholly or incompletely understood. Most individuals with this devastating neuropsychiatric illness benefit from long-term pharmacotherapy, however, the therapeutic effects of antipsychotic treatment are inconsistent, incomplete, and often countered by significant side-effects associated with long-term physical morbidity (e.g., tardive dyskinesia, obesity, hyperglycemia, hyperlipidemia), and subjectively unpleasant states that may lead to nonadherence. Moreover, although most individuals respond to treatment, poor or partial response is common and many individuals require trials of multiple medications.
Methods: Here I discuss prospects for improving the use of antipyschotics using pharmacogenetics, focusing on a study of how variation in over 100 candidate genes influence both efficacy and safety of second-generation antipyschotics. Each of these genes is SNPTagged to capture haplotype diversity and genotyped using Illumina arrays across a large dataset of schizophrenia patients collected across multiple centers.
Results: Array-based genotyping yielded high resolution, complete haplotype information capture for the candidate genes, to be used versus treatment response and side effects of these drugs. The results include a combined candidate-locus and haplotype tagging approach covering over 100 genes. A proof-of-principle finding was made using a very similar approach, in large populations of epilepsy patients. A functional locus in the KCN1 potassium channel gene responsible for variation anti-epileptic treatment response was identified.
Discussion: Investigation of the genetic basis of treatment response in schizophrenia, and other pyschiatric disorders, is essentially in its earliest stages. Very few candidate genes have been adequately evaluated, and genome-wide or system-wide analyses are lacking. The approach offered here to use haplotype-based analysis in a large panel of candidate genes to detect effects of moderately abundant alleles of moderate effect size represents a beginning.

Identification of Genes Influencing Citalopram Treatment Response in the STAR*D Trial

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Background: Responses to selective serotonin reuptake inhibitors (SSRIs) by subjects with mood disorders is variable, with genetic factors contributing in part to treatment outcome. The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial compares treatment options for individuals with major depressive disorder (MDD) who did not respond favorably to citalopram.
Methods: Study subjects, ages 18-75 years, with a baseline Hamilton Rating Scale for Depression score ≥14 meeting DSM-IV criteria for single

or recurrent non-psychotic MDD were eligible. DNA samples from 1380 participants were genotyped using > 900 SNPs selected on prior evidence of association with antidepressant outcome, association with mood disorders, and known function. High throughput genotyping was performed by Illumina, Inc., using a BeadArray platform, while two moderate throughput methods were employed based on Applied Biosystems 5' nuclease and SNPlexTM 48-plex assays. Tests of association included the Pearson chi-square test, likelihood ratio chi-square test, and Fisher's Exact Test (allelic associations only). For the serotonin transporter gene (SLC6A4) promoter polymorphism (HTTLPR), association was also tested based on information that the locus is functionally triallelic.
Results: We detected significant and reproducible association between treatment outcome and a marker in the serotonin receptor 2A gene (HTR2A, rs7997012; p = 1 x 10-6 to 3.7 x 10-5 in the total sample). Another marker in an ionotropic glutamate receptor gene, GRIK1, also showed evidence of association with treatment outcome. In performing the HTR2A analysis, two categorical outcomes (response and remission), and a quantitative outcome based on relative change in the final symptom score were selected. When the total sample was divided by race into "white" and "black" groups, we noted that the "A" allele of rs7997012, that was associated with better treatment outcome in the total sample, was over 6 times more frequent in the white than in the black participants (allele frequency in whites = 0.42, in blacks = 0.06). Second, the association between HTR2A and treatment outcome was largely confined to the white participants. Also of interest, we observed no evidence of association between any of the four SLC6A4 markers genotyped in the total sample and treatment response or remission, although the efficacy of SSRI action has been previously associated with polymorphisms in SLC6A4. To pursue this result further, we obtained HTTLPR genotypes in the total sample, including a common functional A>G variation within the L allele. A significant association with tolerability was observed in the white sub-sample based on LA allele frequency ($\chi 2 = 7.42$, $P = 0.006$) and genotype frequency ($\chi 2 = 7.18$, $P = 0.028$). No significant association was observed between treatment response or symptom remission outcomes and HTTLPR by genotype or allele frequencies.
Discussion: The association between HTR2A and treatment outcome was largely confined to the white participants, suggesting that genetic variation in HTR2A may help explain racial differences in antidepressant treatment outcomes. In addition, HTTLPR polymorphism was linked to citalopram tolerability. Because the LA allele predicts increased transcription of SLC6A4, increased serotonin transporter levels in brain and other tissues may lead to increased tolerance to SSRIs.

Does a Common Variant of the mu Opiate Receptor Gene Predict Response to Naltrexone in the Treatment of Alcoholism? Results from the COMBINE Study
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Background: It has been previously reported that a genetic variation (A118G) in the mu opiate receptor (OPRM1) which codes for an asparagine to an aspartate substitution at position number 40 (asn40asp) causes greater beta endorphin binding and function and may predict greater treatment response to the opiate antagonist naltrexone in alcoholics (Oslin et.al. Neuropsychopharmacology 2003). The COMBINE Study recruited 1383 alcohol dependent individuals into a multisite randomized placebo controlled trial of naltrexone (100 mg),acamprosate (3 grams),or their combination over 16-weeks in the context of either medical management (MM) alone or combined with a specialist delivered behavioral intervention (CBI). As part of the goals for this study,blood was collected for genetic evaluation of treatment response. The first gene evaluated was the OPRM1 and its ability to predict response to naltrexone.
Methods: After informed consent,whole blood was collected and centrally stored prior to DNA extraction and typing for the asn40asp allelic variant and various haplotypes of the OPRM1 gene (taqman assay at NIAAA-LNG). Since the asn40asp polymorphism exists in very low frequency in African Americans, and the initial observation on differential naltrexone response was confined to Caucasians, the present investigation was limited to only Caucasians who received naltrexone (n= 301) or placebo (n=303),irrespective of whether they received acamprosate or not. The frequency of having at least one asn40asp allele was similar between treatment groups (22% in the naltrexone group and 22% in the placebo group) consistent with expected frequencies based on previous reports.
Results: Initial analyses were planned a priori to replicate the analyses previously reported (Oslin et.al.2003). However,since naltrexone was found to exert a differential effect over placebo only in those treated with MM subsequent analyses were confined to individuals who received naltrexone (n=114) vs placebo (n=265) in the context of medical management alone. The biggest effect size for naltrexone was observed using a clinical measure of outcome (whether or not a person was drinking heavily with problems or not during the last 8 weeks of treatment). Using this most sensitive measure of outcome 96% of those individuals treated with naltrexone plus MM who had the asn40asp variant (n=28) had good clinical outcome while only 73% of those without this variant (n=86) had good clinical outcome. Good clinical outcome was observed in 63% asn40asp variant placebo-treated individuals (n=60) vs 65% for those without this variant treated with placebo (n=205. There was no main effect of having the asn40asp allele on good clinical outcome but the interaction between having the allele and response to naltrexone was significant (p=0.03) with an odds ratio of good response to naltrexone in asn40asp variant compared to non-variants of 10.25 (95% CI 1.31-80.0, p=0.03). This interaction was not observed when using more traditional outcome measures, such as relapse to a single heavy drinking day during the study, or percent days abstinent. Haplotypes of the OPRM allele will be further analyzed and presented to evaluate whether better prediction of naltrexone treatment response over and above the single asn40asp polymorphism could be observed.
Conclusions: The results of this large multisite study confirm that for at least one clinically salient outcome measure that the asn40asp coding variant (A118G) of the OPRM gene might have substantial predictive value for naltrexone response and be clinically useful in the treatment of alcohol dependence.

Pharmacogenetics of Antipsychotic Drug Response: From Candidate Gene to Whole Genome Approaches
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Background: Pharmacogenetic studies of psychotropic drug response have primarily focused on simple phenotypic indices such as arbitrary response/non response classifications, and utilized single or limited SNP genotyping due to limitations in power and molecular technology. The next generation of pharmacogenetic studies, however, is now underway with new approaches involving novel phenotypes and more comprehensive genotyping technologies.
Methods: We have utilized several novel phenotypes in pharmacogenetic studies of schizophrenia. First, we will focus on the use of treatment-naïve patients in examining genetic factors influencing speed of therapeutic response. Second, we will discuss recent work aimed to identify genetic predictors of antipsychotic-induced adverse events, including clozapine-induced agranulocytosis. Finally, we have conducted a whole genome association study of schizophrenia utilizing the Affymetrix 500K microarray in a cohort of 400 subjects characterized for diagnosis, symptom profile, neurocognitive function, and treatment response parameters.
Results: In initial candidate gene work, we report that promoter region variation in the DRD2 gene is a significant predictor of therapeutic efficacy in treatment – naïve subjects. Second, we will provide data on five genes, included two genes within the HLA system, with evidence

for association with risk for development of clozapine-induced agranulocytosis. Finally, we will present the results of a whole genome association study of schizophrenia, including our initial data on the genomic regions influencing neurocognitive function and treatment response.
Discussion: Taken together, results from these studies indicate that genetic factors may significantly influence antipsychotic treatment response and development of important drug-induced side effects. The next step is to validate these results in larger scale work and refined neurobiological studies, as well as to continue to execute comprehensive genomic studies. Finally, data from the whole genome association study, albeit preliminary, may provide new, heretofore unrecognized, targets for pharmacogenetic studies in schizophrenia.

Panel Session
New Experimental Approaches to Treatment of Schizophrenia

Allosteric Potentiators of mGluR5 as a Novel Approach to Treatment of Schizophrenia and Other Cognitive Disorders

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Background: Clinical and basic studies have led to the hypothesis that novel compounds that potentiate the function of NMDARs may ameliorate the symptoms of schizophrenia. Furthermore, a number of recent studies suggest that one of the metabotropic glutamate receptor (mGluR) subtypes, mGluR5, is a closely associated signaling partner with the NMDAR and may play an integral role regulating and setting the tone of NMDAR function in a variety of forebrain regions. Based on this, we have postulated that activators of mGluR5 may provide a novel approach that could be useful in treatment of schizophrenia. Unfortunately, it has been difficult to develop selective orthosteric agonists that selectively activate mGluR5 and have properties that are desirable in drug-like molecules. However, we have now developed multiple series of compounds that act as novel and selective positive allosteric modulators (allosteric potentiators) of mGluR5. Studies with these novel compounds suggest that allosetric potentiation of mGluR5 may offer an exciting new approach to treatment of schizophrenia and other disorders that involve impaired cognitive function.
Results: Four distinct structural classes of mGluR5 allosteric potentiators have now been identified. These include three classes discovered by our group and a forth by workers at Addex Pharmaceuticals. None of these compounds activates mGluR5 when added alone but each potentiates activation of human and rat mGluR5 by glutamate and other agonists. These compounds do not alter [3H]-quisqualate binding to the glutamate binding site, but three of the four classes competes for [3H]-methoxyPEPy binding to a site for this allosteric antagonist. Extensive studies suggest that binding to this site is critical for allosteric potentiator activity. We have now used these compounds in electrophysiology and behavioral studies to test the hypothesis that they potentiate mGluR5 activity in circuits that may be important for schizophrenia and cognitive function. Consistent with studies in cell lines, mGluR5 positive allosteric modulators potentiate electrophysiological effects of mGluR5 activation in different populations of forebrain neurons. Interestingly, these compounds also enhance synaptic plasticity in the hippocampal formation. Finally, a series of in vivo studies suggest that allosteric potentiators of mGluR5 have an antipsychotic-like profile in animal models. We are now investigating the effects of these compounds in models of cognitive function that may relevant to cognitive impairments that occur in schizophrenic patients.
Conclusion: These studies raise the exciting possibility that allosteric potentiators of mGluR5 may serve as a novel approach to increasing activity of this receptor for treatment of CNS disorders such as schizophrenia and other disorders that involve impaired cognitive function. We are now gaining tremendous insights into the molecular actions of these compounds, their effects on different electrophysiological responses and signaling pathways, and their actions in vivo. In addition to mGluR5, we have also made progress in discovery and characterization of allosteric potentiators of several other GPCRs. Among these are muscarinic acetylcholine receptors and mGluR2. Interestingly, activity of these compounds and previous studies suggest that allosteric potentiators of these receptors may also provide novel approaches to treatment of schizophrenia. Supported by NIMH, NINDS, NARSAD, The Stanley Foundation. Vanderbilt is a site in the NIH-supported Molecular Libraries Screening Center Network (MLSCN).

Progress Towards Validation of the NMDA Hypofunction Hypothesis of Schizophrenia: Discovery and Development of Centrally Active Glycine Transporter (GlyT1) Inhibitors

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This presentation will discuss recent progress towards validation of the N-methyl-D-aspartate (NMDA) receptor hypofunction hypothesis of schizophrenia in preclinical models. Schizophrenia, a complex disease composed of positive, negative and cognitive symptoms, affects 1% of the world population and requires lifelong, daily maintenance therapy. Historically, treatment for schizophrenic patients has centered on D2 dopamine receptor antagonists, derived from the "Dopamine Hypothesis", which have a slow onset of action and improve only the positive symptoms of the disease. The NMDA receptor antagonist PCP has been shown to induce the positive, negative and cognitive symptoms of schizophrenia in healthy patients and cause a resurgence of symptoms in stable patients. These observations led to the NMDA receptor hypofunction hypothesis as an alternative theory for the underlying cause of schizophrenia. According to this hypothesis, any agent that can potentiate NMDA receptor currents has the potential to ameliorate the symptoms of schizophrenia. To date, NMDA receptor currents can be modulated by either direct action on modulatory sites on the NMDA receptor (i.e., the glycine co-agonist binding site) or indirectly by activation of G-protein coupled receptors (GPCRs) known to potentiate NMDA receptor fucntion (i.e., mGluR5). I will discuss the NMDA receptor hypofunction hypothesis, the NMDA receptor as an emerging target for the development of novel antipsychotic agents and progress towards in vivo target validation with GlyT1 inhibitors. A high-throughout screen of our corporate collection afforded a number of attactive hits as potential leads for our GlyT1 program. Employing parallel optimization and an iterative analog library approach, potent(< 10 nM) and selective (>20 uM versus GlyT2) GlyT1 inhibitors were rapidly prepared. This presentation will describe the discovery and development of this novel series of centrally active, non-sarcosine-derived GlyT1 inhibitors. By microdialysis, compounds within this series selectively increased basal glycine levels in the prefrontal cortex 340% and enhanced prepulse inhibition (PPI) in DBA/2J mice, a behavioral model in which well characterized antipsychotic drugs show simialr positive effects. These data provide strong support for continuing drug discovery efforts aimed at validation of the NMDA hypofucntion hypothesis of schizophrenia.

New Directions in the Treatment of Schizophrenia: Modulators of mGlu2 and/or mGlu3 Receptors

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Glutamate is the major excitatory neurotransmitter in the mammalian CNS responsible for mediating excitation in the majority of

synapses and also plays a key role in long-term processes such as synaptic plasticity which determine the brain's ability to adapt and function. At a systems level glutamate neurotransmission participates in information processing in higher centers, including the acquisition / retention of experience and memories. In spite of the introduction of atypical antipsychotics and dopamine stabilizers, disability from schizophrenia is still a very high unmet medical need and thus exploration of these novel mechanisms may be the key to better understanding of the illness as well as future therapeutic advances. Schizophrenia is a complex disorder that has components of altered sensory gating, emotional reactivity, and disrupted plasticity which manifests positive, negative, and cognitive symptoms. Glutamatergic drugs have potential to treatment certain features of the illness depending on which processes are involved and the circuits that specific agents might correct. Evidence favoring this hypothesis includes the observation that drugs such as phencyclidine and ketamine which act via blockade of NMDA ion channel receptors for glutamate disrupt glutamate neurotransmission and exacerbate psychosis in schizophrenic patients. Metabotropic glutamate (mGlu) receptors (mGlu1-8) are coupled to G-proteins and, depending their expression and subtype, function to regulate glutamate neurotransmission via presynaptic (control of the release of glutamate and other neurotransmitters), post-synaptic (short and long-term regulation of postsynaptic excitability), and glial (production of second messengers that regulate glial function and expression of receptors, transporters and glial factors) mechanisms. With regard to the potential to treat schizophrenia, agonists for mGlu2/3 receptors and allosteric potentiators of mGlu2 receptors block the effects of psychomimetics (such as PCP, amphetamine, 5HT2A agonists) in animals and/or humans. These agents appear to work by preventing glutamatergic hyperexcitations in limbic circuits that has been associated with the actions of psychotogens and possibly in the symptoms of schizophrenia. In essence they will be useful to test the clinical relevance of glutamate hyperexcitability to the etiology of schizophrenia. Their usefulness either alone or in combination with other agents such as atypical antipsychotics needs further study in animals and possibly humans. Overall, the effectiveness and long-term safety of all these agents needs further exploration in animals and humans. Nevertheless, these mGlu receptor active agents represent novel approaches for schizophrenia treatment in a field where monoaminergics are essentially the only clinically useful options for schizophrenia patients currently. This presentation will cover the recent progress, challenges, and issues for the discovery and development of mGlu2 and/or mGlu3 receptor active agents to treat schizophrenia.

Muscarinic Receptor Activation for the Treatment of Schizophrenia

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Cholinergic neurotransmission in the central nervous system (CNS) plays an important role in cognitive processes such as memory and attention. Acetylcholine predominantly exerts its central effects through its interaction with muscarinic acetylcholine receptors (mAChR). Although the involvement of muscarinic receptors in multiple central and peripheral functions has long been known, it is only recently that the physiological contribution of each subtype has been described following the generation M1-M5 knock out mice (for review see Birdsall et al., 2001; Bymaster et al., 2003). Interestingly, transgenic mice lacking M1 receptors, the predominant CNS muscarinic receptor, exhibited deficits in cortical MAPK signaling (Hamilton and Nathanson, 2001), hippocampal long-term potentiation and memory processes involving cortico-hippocampal interactions (Anagnostaras et al., 2003). Furthermore M1 deficient mice showed impairment in dopaminergic neurotransmission and a concomitant hyperactivity phenotype (Miyakawa et al., 2001; Gerber et al., 2001). The sensitivity of this phenotype to typical and atypical antipsychotic treatment supports a role for M1 receptors in psychiatric disorders involving a dysfunction of the dopaminergic system such as schizophrenia (Gerber et al., 2001). Consistent with this proposal, clinical observations have demonstrated psychotomimetic effects of muscarinic antagonists in humans (Gershon, 1960; Neubauer, 1996) and notably a double-blind, placebo controlled clinical study with M1/M4 agonist xanomeline in Alzheimer's disease patients revealed significant improvements in psychotic behavior and cognitive abilities (Bodick et al., 1997). Moreover, preclinical studies have illustrated the antipsychotic profile of xanomeline (Shannon et al., 2000; Stanhope et al., 2001). Although M1 agonists clearly have potential for the treatment of psychosis, dementia and other disorders, successful development of highly selective M1 agonists has been difficult due, in part, to the high level of amino acid conservation of the acetylcholine (ACh) orthosteric site between the five muscarinic subtypes (Hulme et al., 1990; Wood et al., 1999). Muscarinic receptor ectopic agonist approaches have been proposed in order to circumvent this issue (Lazareno et al., 1998; Spalding et al., 2002; Christopoulos, 2002). The feasibility of this approach has recently been exemplified by the discovery of the ectopic agonist AC42 (Spalding et al., 2002). This small molecule is a modestly potent partial agonist at human M1 receptor with high selectivity for this subtype. Furthermore, pharmacological and site-directed mutagenesis studies clearly demonstrated that AC42 does not mediate its agonist activity by interacting with ACh orthosteric site but through a novel ectopic site (Spalding et al., 1987). Recent work from our group has further validated the concept of ectopic agonists by showing that the major metabolite of the atypical antipsychotic clozapine, N-desmethylclozapine, is a potent allosteric agonist at human M1 receptors (Sur et al., 2003). We have more recently reported the discovery of a novel M1 allosteric agonist and associated in vitro pharmacological and electrophysiological studies. Behavioral studies further revealed the activity of ectopic agonists in animal models sensitive to antipsychotic drug treatment and support the view that selective M1 agonists may represent a novel therapeutic approach for the treatment of psychotic disorders.

Panel Session
Prefrontal-Accumbens-Amygdala Circuitry in Impulsive Aggression: Implications for Treatment Development

Behavioral Changes Following Selective Lesions of the Amygdala, Hippocampal Formation, and Orbital Frontal Cortex in Monkeys

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Background: Social interactions are a key component of primate behavior and require the use of specific skills that combine to efficiently regulate behavioral selection towards achieving a social goal, i.e. recruiting an ally, attracting a mate, etc. While the neural network that mediates such complex cognitive skills is not completely understood, two brain structures, the amygdala and orbital frontal cortex, appear to be critical for efficient social cognition in several species, including humans. Although early lesion studies in monkeys have shown that both structures are critically involved in the maintenance of normal social interactions, the lesions were generally large and not restricted to specific structures, observational measures were not sophisticated and behaviors of the animals were never investigated pre- and post-surgery. Thus, to gain further knowledge of the respective contribution of the amygdala, hippocampal formation and orbital frontal cortex to social cognition, the present study used selective lesion techniques, a semi-naturalistic social environment, as well as ethologically-valid and detailed behavioral observations of macaque monkeys to compare and contrast the effects of selective damage to these 3 neural structures on the maintenance of previously established social relationships, taking into account the pre-surgical social rank (dominant versus subordinate) of the animals.

Methods: Social dominance, personality ratings, as well as frequency, duration and timing of social behaviors were measured pre- and post-surgically in 6 groups of male monkeys, each consisting of 1 sham-operated control, and 1 animal each with selective amygdala, hippocampal or orbital frontal cortex lesion, during 1-hour social interactions across 15 days. Thus, each group consisted of 6 animals each.
Results: Unlike previous reports, none of the animals showed significant changes in social dominance status post-surgery, although changes in all other behavioral measures varied according to lesion site. While sham-operated animals displayed heightened avoidant, anxious and aggressive behaviors, those with hippocampal lesions also showed increased exploratory behaviors and excitability, and reduced responses to affiliative signals. Amygdala lesions yielded several personality changes that do not promote positive social interactions (increased excitability and cage exploration; decreased affiliation and popularity) and altered responses to threatening social signals. By contrast, animals with orbital frontal lesions displayed more aggressive gestures, received more contact aggression, and responded differently to both affiliative and threatening signals. The findings, which are not entirely consistent with those obtained in earlier studies, emphasize the context-specific nature of the behavioral changes observed and largely parallel those reported in recent human studies. Interpretations of the results in relation to cognitive processes known to be mediated by the amygdala, orbital frontal cortex, and hippocampus will be discussed.

Neurocircuitry of Aggression in Borderline Personality Disorder and Its Implications for a Rational Approach to Pharmacology
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Background: Patients with borderline personality disorder (BPD) frequently suffer from impulsive aggression (IAD), 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 studies employing a serotonin agonist showed that BPD patients with IAD exhibit less activation in the OFC and ACG than healthy controls. The present study extends this research by using ^{18}FDG-PET scans during an anger provocation task, the Point Subtraction Aggression Paradigm.
Methods: 27 healthy controls and 38 age- and sex-matched BPD-IAD patients completed the PSAP during ^{18}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: In preliminary analyses of the ACG, rGMR was entered into a mixed-design ANOVA consisting of group (BPD, HC) 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. rGMR in Brodmann Areas in ACG (BA 25, 24) correlated positively with aggressive behavior on the PSAP in controls and negatively with aggressive button pressing in patients. For the OFC, rGMR was entered into a diagnostic group (BPD, HC) 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 no significant interaction involving provocation condition was detected. This indicates decreased OFC activity in BPD-IAD patients compared to controls regardless of provocation condition. We also noted that patients showed aggressive responding during both the provocation and non-provocation condition, while controls responded aggressively only in the provocation condition as expected.
Conclusion: These findings demonstrate that BPD-IAD patients respond aggressively even without provocation. It also shows **decreased** rGMR in OFC in patients compared to controls regardless of provocation condition, and **decreased** activation in ACG during the provocation condition while normal controls show **increased** activity specifically in response to provocation. These findings suggest specific deficits in areas of the prefrontal cortex implicated in emotion processing in response to aggression provocation in aggressive patients with borderline personality disorder. Our findings of decreased activity in OFC and ACG in impulsive aggressive BPD patients led us to evaluate the efficacy of a pharmacological agent that increases activity in prefrontal cortex, guanfacine, as a therapeutic intervention for BPD-IAD and preliminary evidence of symptom change will be reported.

Reward Processing and Pharmacological Dissection in Non-Aggressive Impulse Control Disorders
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Background: The ICDs may be divided into aggressive (IED) and non-aggressive (Pathological Gambling (PG), Trichotillomania, Pyromania, Kleptomania) subtypes. Prefrontal-accumbens-amygdala circuitry has been implicated in the modulation of impulsivity, and experimental therapeutics for this symptom domain may target modulation of this circuitry.
Methods: A Siemens 3T fMRI study of anticipatory (motivational) and outcome (consummatory) reward processing via the Modified Monitary Incentives Delay (MID) task subjects was conducted in 6 right-handed PG vs 6 healthy subjects. Seven PG subjects also carried out a computer blackjack task following injection of FDG under two different reward conditions: monetary reward and computer game points only. Relative FDG metabolic rate was obtained from regions of interest in the prefrontal cortex, cingulate, striatum and visual cortex. Three placebo controlled trials of lithium (N=40), topiramate (N=43), and nalmefene (N=206) were conducted in adult outpatients with nonaggressive PG.
Results: All subjects showed increased activation in the ventral striatum when anticipating responding to monetary incentives vs no monetary incentive; however, PG subjects evidence less activity than controls in nucleus accumbens when anticipating responding to rewards. In contrast, there was no group difference in response to receiving rewards. Monetary reward blackjack was associated with significantly higher relative metabolic rate in the cingulate gryus (Brodmann area 24), the putamen and prefrontal areas 47 and 10, compared to blackjack playing for points only. Lithium, topiramate and nalmefene reduced thoughts/urges and impulsive behavior in nonaggressive PG.
Conclusion: Underactivity of the Ventral Striatum may predispose subjects to engage in high risk behaviors. Heightened limbic activation in the gambling for money condition with increased emotional valence and greater risk and reward confirms the salience of monetary reward in the development of PG. Accumbens underactivity, Amygdala activation, and prefrontal hypofunction may predispose subjects to non-aggressive impulsivity. Glutamatergic, second messenger, and opiate agents which modulate this circuitry may target non-aggressive impulsivity.

Cortico-Limbic Circuits in IED: Insights from fMRI Studies
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Intermittent Explosive Disorder (IED) is a relatively common disorder (4-5% lifetime prevalence in the general population) of recurrent,

problematic, impulsive aggression. In addition to reductions in central serotonergic function, subjects with IED also display deficits in social-emotional information processing. fMRI studies in our laboratory reveal that, compare with healthy controls, subjects with IED display enhanced BOLD responses of the amygdala in response to exposure to harsh emotional faces; specifically faces of anger. Examination of orbitomedial prefrontal cortex (OMPFC) activity in the same tasks reveals greater activity in this region in healthy controls compared to subjects with IED. In addition, connectivity analyses demonstrate significant correlations between amygdala and OMPFC activity in response to anger faces in healthy volunteers but not in subjects with IED. These data indicate clear dysfunction in social-emotional information processing in subjects with IED copmpared with healthy controls. Analysis of data of these fMRI parameters examined in subjects with IED both before and after treatment with antiaggressive agents (i.e., fluoxetine or divalproex) will, for the first time, also be discussed in this presentation.

Panel Session
Synapse Failure in Dementia: How Much is Functional?

Endosome Dysfunction in Alzheimer's Disease: Genetic Links and Implications for Synapse Failure and Neurodegeneration

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Endocytosis is universally important in cell function. In brain, the roles of endosomes are relatively more complex due to the unique polar shape of neurons and the specialized needs for long distance signaling and communication at synapses. These needs, which include synaptic vesicle recycling, receptor modulation, neurotrophin signaling, and membrane trafficking, make the brain particularly vulnerable to a disturbance of endocytic trafficking. Underscoring the special vulnerability of neurons are recent findings that causative gene mutations or risk-enhancing gene polymorphisms in at least five different degenerative diseases associated with dementia influence endosome regulation. In Alzheimer's disease (AD), many proteins and factors that influence pathogenesis converge at the endosome, including the amyloid precursor protein (APP) and its processing enzymes, Aβ peptide, cholesterol, apolipoprotein E (ApoE), and other key lipoprotein-regulating proteins. Alterations of endosome morphology, which are the earliest known specific pathology in AD, precede β-amyloid deposition and coincide with the first appearance of intra-endosomal Aβ in neurons. Microarray profiling of individual neurons at this very early stage of disease reveals selective changes in the expression of genes regulating endocytosis, supporting immunological and functional evidence from brain and cell models for altered endocytic trafficking in AD. Endosomes are a major site for Aβ generation and cells modeling AD-related endosome pathology over-produce Aβ. Endosome pathology is accentuated in individuals who inherit the ApoE epsilon-4 genotype, a major genetic risk factor for AD, and it begins to develop at very early ages in individuals with Down Syndrome (DS,Trisomy 21), a cause of early-onset AD involving App triplication. In a mouse model of DS (Ts65Dn), endosomal pathology similar to that in AD and DS develops in neurons in cholinergic circuits that later exhibit aging-related degenerative changes. Endosome pathology in Ts65Dn mice specifically requires triplication of the App gene, consistent with recent genetic evidence that App duplication causes an early-onset form of AD. Further analyses using a small interfering RNA approach are identifying how APP gene dosage drives endosome dysfunction. Additional ongoing studies have provided insight into the impact of presenilin mutations on the fate of endosomal traffic from synaptic terminals. Our findings show that genes related to the development of AD have major effects on endosome regulation, supporting a close relationship between endosome dysfunction and AD pathogenesis. Defective neuronal endocytosis has important implications for synaptic transmission, retrograde signaling, and Aβ overproduction, each of which contributes to degeneration of specific neural circuits and dementia. Supported by NIA.

Increased APP Gene Expression in a Mouse Model of Down Syndrome Disrupts Retrograde Endosomal Transport of NGF and Causes Cholinergic Neuron Degeneration

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Background: A key element of endosomal biology is the long distance transmission of neurotrophin signals from axon terminals to cell bodies. Endosomes that contain the internalized neurotrophin bound to its receptor – so-called 'signaling endosomes'- constitute an important means for such signals. Following their formation they are moved retrogradely within axons by dynein on microtubules. Studies to examine and characterize signaling endosomes are providing an exciting new perspective on neurotrophin actions. Equally important, these studies point to the possibility that failed trafficking of signaling endosomes may contribute to neurodegeneration. We will review basic features of endosomal signaling. We will then review recent studies examining the link between disrupted transport of signaling endosomes and degeneration of basal forebrain cholinergic neurons. Dysfunction and loss of these neurons contributes to cognitive dysfunction in Alzheimer's disease (AD) and Down syndrome (DS).
Methods: We used the Ts65Dn and Ts1Cje mouse models of DS to examine NGF retrograde transport and the status of cholinergic neurons of the basal forebrain. For transport studies, radiolabelled NGF was injected into hippocampus; radioactivity was measured in the septum 12 hours later. Stereological methods were used to examine the size and number of cholinergic neurons in the medial septal nucleus. App gene dose was correlated with increased expression in these mice.
Results: We showed in mouse models of DS that the increased dose of the amyloid precursor protein gene, App, acts to markedly decrease retrograde transport of Nerve Growth Factor (NGF) and causes degeneration of cholinergic neurons. NGF transport in cholinergic neurons was also decreased in mice expressing wild-type human APP or an FAD-linked mutant APP. Because the transport defect was intra-axonal, we explored within cholinergic axons the status of early endosomes, a compartment known to retrogradely transport NGF. NGF-containing early endosomes were enlarged in Ts65Dn mice and the content within early endosomes of App was increased, thus directing attention to this compartment as one possible locus for failed NGF transport.
Conclusion: Our studies provide evidence for a pathogenic mechanism for DS in which increased expression for App, in the context of trisomy, causes abnormal retrograde transport of NGF within signaling endosomes. We speculate that failed NGF retrograde signaling is responsible for cholinergic neurodegeneration.

AMPA-R Removal Underlies Aβ-induced Synaptic Depression and Dendritic Spine Loss

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Beta amyloid (Aβ), is a peptide generated by neurons and widely believed to underlie the pathophysiology of Alzheimer's Disease when overproduced. Recent studies indicate this peptide can drive loss of surface AMPA and NMDA type glutamate receptors. We now show that Aβ employs signaling pathways of LTD to drive endocytosis of synaptic AMPA receptors. Synaptic removal of AMPA receptors is key, as it is necessary and sufficient to produce loss of dendritic spines and synaptic NMDA responses. Our results indicate that increased

levels of Aβ tap into endogenous physiological processes to depress synaptic structure and function.

Neurotransmitter Release and Neurodegeneration
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At a synapse, release of neurotransmitters initiates synaptic transmission. Our laboratory is interested in how a presynaptic terminal executes release in a tightly regulated and topologically restricted manner. Neurotransmitter release occurs by synaptic vesicle exocytosis that is triggered by influx of Ca2+ into the presynaptic terminal. Ca2+-evoked synaptic vesicle exocytosis is one of the fastest and most tightly controlled reactions in biology, and can be modulated during synaptic plasticity. The speed and plasticity of neurotransmitter release are one of the major factors that shape the exquisite precision of synaptic networks. In a longstanding program, we are investigating the molecular cascades that orchestrate synaptic vesicle exo- and and endocytosis. Hundreds, maybe thousands of proteins are involved in release. We envision that these proteins mediate release in a hierarchy of reactions: At the lowest level of this hierarchy, release is effected by exocytosis. Exocytosis in turn is controlled by Ca2+, and the Ca2+-dependent release machinery is embedded in the active zone of the presynaptic terminal by a protein scaffold that integrates signalling during synaptic plasticity. At the top level of the hierarchy, a transsynaptic cell adhesion apparatus organizes the position and activation of the release machinery with respect to the postsynaptic specializations. We are trying, in conjunction with other laboratories, to achieve a systematic description of the molecular components that mediate neurotransmitter release, and to analyse these components structurally and functionally. Studies in our and other laboratories have identified major players at each level of the presynaptic hierarchy. Exocytosis is largely mediated by SNAREs (e.g., synaptobrevin, syntaxin and SNAP-25) and SM proteins (e.g., Munc18-1), and is controlled by synaptotagmins as Ca2+-sensors. Key components of the protein scaffold of the active zone, such as Munc13s, RIMs, and ELKS integrate synaptic vesicle exocytosis and mediate synaptic plasticity. An initial, sketchy description of the molecular machinery that executes neurotransmitter release has thus emerged, a description that hopefully will form the basis for a mechanistic understanding of this central process in synaptic transmission. An unexpected recent discovery in these studies were observations that tie the synaptic machinery which mediates vesicle exo- and endocytosis to neurodegeneration. Two specific findings suggested new and unexpected connections between the fusion machine of synaptic vesicles and neurodegeneration. The first finding shows that the nerve terminal contains proprietary chaperones which keep vesicle exo- and endocytosis alive over the lifetime of an organism; interference with these chaperones causes massive neurodegeneration that is linked to synucleins which are involved in Parkinson's disease. The second finding reveals a role for synaptic trafficking of APP, a key molecule involved in Alzheimer's disease, in neuronal signalling. In my talk, I will discuss these new findings, and highlight in particular mechanisms of neurodegeneration that involve dysfunction of chaperones acting on SNARE proteins, and that may contribute to common diseases such as Alzheimer's and Parkinson's disease.

Panel Session
The Vesicular Monoamine Transporter (VMAT) as a Target for Novel Therapeutics

Pharmacological and Clinical Implications of VMAT2
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Brain vesicular monoamine transporter 2 (VMAT2) has a critical role in the regulation of monoaminergic neurotransmission.We have shown that the expression of this protein can be regulated by steroid hormones, pharmacological agents and drugs active at the monoaminergic system as well as by stress, anxiety and mental illnesses. Rat brain VMAT2 expression is suppressed by chronic progesterone and estradiol administration. These modulatory effects may be relevant to synaptic and neuronal plasticity and may have a role in gender-specific neuropsychiatric disorders. Decreased VMAT2 levels were found in both genetic rat model of depression and following swim stress, a laboratory model for chronic anxiety and depression. On the pharmacological level, we demonstrated that the antipsychotic clozapine, but not haloperidol affects rat brain VMAT2. This phenomenon may be ascribed to the unique therapeutic advantages of clozapine in schizophrenia. The classical mood stabilizer lithium increased VMAT2 expression in rat prefrontal cortex, a modulatory effect that can be implicated in mood stabilization and antidepressive activity of this agent. Rat brain VMAT2 expression is modulated by addictive agents such as psychostimulants and methadone. On the clinical level, we found alterations in platelet VMAT2 density in patients with major depression, schizophrenia, social phobia and ADHD, disorders related to dysregulation of the monoaminergic system. In addition, changes in platelet VMAT2 expression were noted in habitual smokers and heroin addicts maintained on methadone. Our studies demonstrate the involvement of VMAT2 in the organism response to monoaminergic agents and monoamine-related psychiatric disorders.

Lobeline Analogs as Potent and Selective VMAT2 Antagonists
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Background: VMAT2 plays an important role in mediating behavioral effects of psychostimulants, and the abuse liability of such drugs is thought to result from modulation of the dopaminergic system in the brain, which is generally accepted to be responsible for the rewarding effects of these abused drugs. Through an interaction with VMAT2, amphetamine and methamphetamine promote dopamine release from synaptic vesicles into the cytosol of the dopaminergic presynaptic terminals. Lobeline, isolated from Lobelia inflata, inhibits both the neurochemical and behavioral effects of amphetamine in rodents, which is believed to be due to a noncompetitive inhibition of VMAT2. Interestingly, lobeline is not self-administered in animals, which is consistent with the fact that lobeline does not evoke dopamine release. The observation that lobeline inhibits methamphetamine-evoked dopamine release from superfused rat striatal slices is consistent with its ability to decrease methamphetamine self-administration. Thus, VMAT2 is a potential target for the development of agents to treat methamphetamine abuse, and novel lobeline analogs that are selective VMAT2 antagonists may have potential as clinical therapies.
Methods: For the α4β2, α7, and α6β2β3* nAChR and VMAT2 binding assays, [3H]-S(-)-nicotine, [3H]-methylycaconitine and [3H]-dopamine, and [3H]dihydrotetrabenazine, respectively, were utilized as radioligands. Rat whole brain (excluding cerebellum) was utilized in the α4β2* and α7* assays, and rat striatal membranes and synaptic vesicles, respectively, were used in the α6β2β3* and VMAT2 assays. The structural characterization of new analogs was carried out utilizing 1H- and 13C-NMR spectroscopy and HPLC-MS analysis. Purity was determined by high resolution mass analysis and elemental analysis. Compounds were evaluated as their water-soluble hydrochloride salts.
Results and Discussion: Lobeline, was found to be a potent, nonselective nAChR antagonist, exhibiting antagonist action at α4β2, α7, and α6β2β3* nAChRs. Lobeline displaced [3H]nicotine binding from native nAChRs with high affinity (Ki = 4-30 nM) and inhibited nicotine-evoked [3H]DA overflow from rat striatal slices (IC50 = 1 μM), suggestive of an antagonist action at α6β2β3* nAChRs mediating DA release. Lobeline also inhibited nicotine-evoked 86Rb+ efflux from rat thalamic synaptosomes (IC50 = 0.7 μM) and inhibited

[3H]nicotine binding to rat striatal membranes (Ki = 4.7 nM), indicating that lobeline is also an antagonist at $\alpha4\beta2^*$ nAChRs. Lobeline also inhibited [3H]methyllycaconitine binding to rat brain membranes (Ki = 6.26 μM), indicating an interaction with $\alpha7^*$ nAChRs, and has been reported to be an antagonist at human $\alpha7^*$ nAChRs expressed in Xenopus oocytes (IC50 = 8.5 μM). In addition to interacting with nAChRs, lobeline inhibits [3H]DTBZ binding to VMAT2 (IC50 = 0.90 μM), and inhibits [3H]DA uptake into rat striatal vesicle preparations (IC50 = 0.88 μM). Therefore, lobeline is a nonselective nAChR antagonist, but also inhibits neurotransmitter transporter function. Several novel synthetic lobelane analogs (i.e. chemically defunctionalized lobeline derivatives) have been identified that were both potent and selective VMAT2 antagonists. These compounds exhibited Ki values at VMAT2 in the range of 400-600 nM and were devoid of nAChR activity. The structure-activity data generated from these studies was utilized in developing a non-linear computational neural network approach to provide training sets for predicting second generation molecules of high affinity and selectivity for VMAT2, and for determining the VMAT2 pharmacophore. Thus, Lobeline represents a novel prototypical molecule from which selective VMAT2 inhibitors can be developed as potential therapeutic agents to treat psychostimulant abuse.

Vesicular Monoamine Transportre 2 (VMAT2) Mice Present a Depressive-Like Phenotype

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The VMAT2 protein transports cytoplasmic monoamines into secretory vesicles and it is found primarily within the CNS. Reserpine blocks VMAT2 transport and produces depressive-like symptoms in some patients. We examined VMAT2 mice for evidence of anxiety- and depressive-like behaviors. VMAT2 knockout mice die soon after birth; heterozygous (HET) animals survive and brain dopamine, norepinephrine, and serotonin are reduced compared to wild type (WT) controls. In tests of anxiety-like behaviors in the open field, light-dark box, zero maze, or novelty-suppressed feeding tests, responses of WT and HET mice are comparable. Thus, HET animals do not appear anxious. However, activity in the open field is reduced in HET animals, suggestive of locomotor retardation. In forced swim, heterozygotes show increased immobility and imipramine normalizes this behavior. Enhanced immobility times are also observed in tail suspension and this is alleviated by fluoxetine, reboxetine, and bupropion. In an anhedonia test, HET mice consume less sucrose solution than WT mice. In learned helplessness, one group of mice freely explores the test apparatus, while the other is given foot-shocks over which they have no control. Upon test, WT animals given prior foot-shock show increased latencies to escape and more escape failures than WT mice previously exposed to the apparatus alone. Hence, the former group shows learned helplessness. Latencies to escape and escape failures are higher in HET than WT animals pre-exposed to foot-shock. Hence, stress enhances learned helplessness in HET mice. Basal serum corticosterone levels are similar in both genotypes; however, concentrations in heterozygotes are more augmented by stress. Together, these findings suggest disruption of a single VMAT2 allele produces a depressive-like phenotype in mice that includes locomotor retardation, behavioral despair, anhedonia, and enhanced behavioral and endocrine responses to stress. [Supported in part by NIH grant MH60451].

VMAT2: Parallels Between Data from Heterozygous Knockout Mice and Data Examining Influences of Imprinted Human Haplotypes

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A decade of studies on heterozygous VMAT2 knockout mice that we and others have created support possible roles for VMAT2 variation in addictive and other drug responses, sleep, aging/neurodegenerative and cardiovascular penotypes. Recent work on the human VMAT2 gene and its common imprinted variants supports methylated-haplotype-dependent alterations in levels of expression that are almost as large as those manifest by heterozygous knockout mice. We describe work that examines effects of human haplotypes that might parallel aspects of these murine phenotypes. We focus on identification of association of imprinted VMAT2 haplotypes in addictive, sleep and drug response phenotypes. These studies support substantial, plieotrophic roles for human VMAT2 variation. They unerscore the benefits taht can flow from careful consideration of mouse knockout data when designing studies to assess the effects of human gene haplotypes.

Panel Session
Transcriptional Regulation of Synaptic Function

Regulation of Neuronal Excitability by the Transcription Factor CREB

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Background: The transcription factor CREB plays an important role in many forms of experience-dependent plasticity. In the nucleus accumbens, it has a particularly critical role in mediating the behavioral responses to drugs of abuse. However, it is unknown what cellular modifications CREB activation causes to mediate its behavioral effects.
Methods: To examine the effects of CREB activation and inhibition on the membrane excitability and synaptic properties of NAc medium spiny neurons (MSNs), we used recombinant Sindbis pseudovirions to express GFP-tagged constitutively active or dominant negative forms of CREB (caCREB and dnCREB, respectively) in slice cultures of the NAc. We also made in vivo stereotaxic injections of viruses into the NAc and prepared standard acute slices from these animals. Whole-cell patch clamp recordings were made from infected and non-infected cells to assay basic electrophysiological and synaptic properties.
Results: Expression of caCREB increased the intrinsic excitability of NAc medium spiny neurons (MSNs) as measured by the number of spikes elicited by depolarizing current injections. Expression of dnCREB had the opposite effect, decreasing the magnitude of evoked spiking. Preliminary analyses suggest that these changes in excitability are due to changes in the numbers and/or properties of sodium and potassium channels. In vivo cocaine administration caused a decrease in NAc MSN excitability, a change that was fully reversed by in vivo expression of caCREB. To directly address the influence of NAc MSN excitability on behavioral responses to cocaine, we manipulated the excitability of these neurons by overexpressing an inwardly rectifying potassium channel subunit, Kir2.1. Compared to animals in which GFP alone was expressed in the NAc, Kir2.1 expression had no effect on basal locomotor activity but dramatically enhanced the increase in locomotor activity in response to acute cocaine (15 mg/kg) administration.
Conclusion: These results demonstrate that CREB activity in the NAc influences the excitability of NAc MSNs and that this physiological adaptation alone is sufficient to alter one extensively studied behavioral response to cocaine. These results also suggest that CREB-induced modulation of the intrinsic membrane excitability of NAc MSNs may contribute importantly to drug-induced plasticity in the brain's reward pathways.

Regulation of Synapse Differentiation by MEF2 Sumoylation

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Postsynaptic differentiation of dendrites is an essential step in synaptogenesis. We have uncovered a requirement for the transcription fac-

tor myocyte enhancer factor 2A (MEF2A) in the differentiation of postsynaptic granule neuron dendritic claws in the cerebellar cortex. MEF2A knockdown in rat cerebellar slices and in the postnatal rat cerebellum significantly impaired the development of granule neuron dendritic claws. Remarkably, a transcriptional repressor form of MEF2A that is sumoylated at Lys403 promoted dendritic claw differentiation. Activity-dependent calcium signaling induced a calcineurin-mediated dephosphorylation of MEF2A at Ser408 and thereby promoted a switch from sumoylation to acetylation at Lys403, leading to inhibition of dendritic claw differentiation. Our findings define a mechanism underlying postsynaptic differentiation that may modulate activity-dependent synapse development and plasticity in the brain.

DNA Methyltransferases in Synaptic Plasticity
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DNA (cytosine-5) methylation represents one of the most widely used forms of enduring cellular memory. Stable patterns of DNA methylation are established during development, resulting in creation of enduring cellular phenotypes. There is growing evidence that the nervous system has co-opted a number of cellular mechanisms used during development to subserve the formation of long-term memory. Moreover, misregulation of DNA methylation is beginning to be implicated in a variety of pshyciatric and behavioral disorders. We examined the role DNA (cytosine-5) methyltransferase (DNMT) activity might play in regulating the induction of synaptic plasticity. We found that the DNA within a region of the reelin promoter, a gene recently discovered to be involved in the induction of synaptic plasticity in the adult hippocampus, exhibited rapid and dramatic changes in cytosine methylation when DNMT activity was inhibited. In addition, DNMT3A gene expression was increased by activation of protein kinase C (PKC) in the hippocampus. Moreover, zebularine and 5-aza-2-deoxycytidine, inhibitors of DNMT activity, blocked the induction of long-term potentiation in the hippocampus. Finally, we found that DNMT activity is required for PKC-induced increases in histone H3 acetylation. Considered together, these results suggest that DNMT activity is dynamically regulated in the adult nervous system, and that DNMT may play a role in regulating chromatin structure and the induction of lasting forms of synaptic plasticity in the mature CNS.

MeCP2-Dependent Transcriptional Repression Regulates Excitatory Neurotransmission
Lisa Monteggia*, Erika Nelson and Ege Kavalali

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Mutations in a single gene, methyl-CpG-binding protein 2 (MeCP2), result in a neurodevelopmental disorder called Rett Syndrome (RTT). This disorder is a pervasive developmental disorder with symptoms including repetitive hand movements, mental retardation, and autistic-like behavior. MeCP2 is a transcriptional repressor that binds to methylated CpG islands in genomic DNA in combination with histone deacetylases to suppress gene expression. Mutations in the MeCP2 gene, which are predicted to result in loss of function of the gene, would then be expected to result in aberrant gene expression although numerous microarray studies have not found altered levels of gene expression. Interestingly, mutations in the MeCP2 gene have also been identified in autism patients that do not meet criteria for RTT. Based on the neurological phenotypes observed in RTT patients, we have taken the approach of investigating whether MeCP2 plays a role in the maintenance and/or regulation of synaptic transmission between central neurons. To examine this, we carried out a number of electrophysiological and imaging experiments on hippocampal neurons cultured from MeCP2 knockout mice. Using electrophysiological recordings, we found a very specific decrease in excitatory transmission in the neurons lacking MeCP2 that appeared to result from a deficit in the presynaptic neuron. In contrast, the frequency of spontaneous inhibitory synaptic currents was unaffected by the loss of MeCP2 suggesting a specificity in the synaptic deficit. To delineate the specific presynaptic alteration producing this functional deficit, we examined different aspects of the presynaptic machinery including the number of presynaptic terminals per dendritic length, the number of readily releasable vesicles, the size of the total recycling pool of vesicles and the kinetics of synaptic depression/recovery. Our findings suggests a role for MeCP2 in presynaptic control of neurotransmitter release and vesicle recycling. To explore whether these functional effects can be attributed to MeCP2's role as a transcriptional silencer, we treated cultures with drug that impairs histone deacetylation and DNA methylation and examined spontaneous synaptic transmission. Treatment with these compounds induced a similar decrease in mEPSC frequency in wild type control cultures but this decrease was occluded in MeCP2-deficient neurons suggesting that these alterations in synaptic neurotrasmission is directly due to MeCP2's role as a transcriptional repressor. Finally, using a lentivirus expressing Cre recombinase, we show that loss of MeCP2 function after neurodevelopment and synaptogenesis was sufficient to mimic the decrease in mEPSC frequency seen in constitutive MeCP2 KO neurons. Together, these studies suggest that this gene may play a critical role in neuronal transmission and alterations in this gene may underlie some of the deficits observed in Rett and Autism patients.

Wednesday, December 6, 2006

Panel Session
Emerging Tools for Alzheimer's Disease Modification Trials

PET Imaging of Brain Amyloid and Tau Deposits
Gary W. Small*, Vladimir Kepe, Linda M. Ercoli, Prabha Siddarth, Susan Y. Bookheimer, Karen J. Miller, Helen Lavretsky, Greg M. Cole, Harry V. Vinters, Paul Thompson, S. C. Huang, N. Satyamurthy, Michael E. Phelps and Jorge R. Barrio

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Background: Amyloid senile plaques and tau neurofibrillary tangles are neuropathological hallmarks of Alzheimer's disease that accumulate in cortical brain regions in amnestic mild cognitive impairment (MCI) patients who are at risk for Alzheimer's disease. Until recently, these abnormal protein deposits could only be assessed at autopsy or rarely, at biopsy.
Methods: We performed positron emission tomography (PET) scans on 60 subjects (20 controls, 20 MCI, 20 Alzheimer's disease) after intravenous injections of 2-(1-{6-[(2-[F-18]fluoroethyl)(methyl) amino]-2-naphthyl}ethylidene)malononitrile (FDDNP), a molecule that binds to plaques and tangles in vitro. All patients also received 2-deoxy-2-[F-18]fluoro-D-glucose (FDG)-PET scans, and 51 received magnetic resonance imaging (MRI) scans. Clinical assessments and FDDNP-PET scans were repeated for 12 subjects (8 controls, 4 MCI subjects) after approximately two years.
Results: Global FDDNP-PET binding (temporal, parietal, posterior cingulate, and frontal average) was lower for the control group compared with the MCI group ($P<0.001$), which showed lower binding than the Alzheimer's disease group ($P<0.001$). FDDNP-PET binding also differentiated subject groups better than did FDG-PET metabolism or MRI volume. Subjects who progressed clinically showed increased FDDNP binding (5 to 11 percent), and autopsy follow-up of an Alzheimer's disease patient demonstrated high plaque and tangle concentrations in brain regions with high FDDNP binding.
Conclusions: FDDNP-PET scanning differentiates MCI from Alzheimer's disease and normal aging. These results point to the potential utility of FDDNP-PET as a potential future surrogate marker

of the efficacy of anti-amyloid or anti-tau treatments, as well as a non-invasive method to determine regional cerebral patterns of amyloid and tau accumulation in living humans.

Functional MRI and Structure-Function Mapping in the Early Diagnosis of Alzheimer's Disease

Susan Bookheimer*, Meredith Braskie, Alison Burggren, Karen Miller, Linda Ercoli, Paul Thompson and Gary Small

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Background: Imaging studies using PET and MRI indicate that brain changes reminiscent of AD occur years and perhaps decades before disease onset. Pathological analysis suggests a progression of plaque deposition beginning in entorhinal cortex and spreading through the hippocampus proper. Prior to the onset of measurable memory decline, FMRI studies of memory have shown abnormal activation, though how these patterns relate to structural abnormalities and memory performance is not understood.
Methods: Twenty-five subjects, 14 older (mean=61.3; range=48-77) and 11 younger adults (mean=29.4; range=25-36) underwent high resolution structural and functional MRI of the medial temporal region to measure cortical thickness and blood flow changes during a memory task. FMRI was conducted at 3T using a gradient echo EPI sequence with 1.5 mm in-plane resolution (3mm slices) perpendicular to the long axis of the hippocampus; structural scans had .4 x .4 x 3 mm resolution. Using anatomical guidelines outlined by Amaral and Insausti (1990) we delineated the right and left sub-regions of the hippocampus and surrounding cortex on the structural scans, focusing on entorhinal cortex (ERC), subiculum, CA1 and an area that included CA2, CA3 and dentate gyrus (CA23DG), and calculated the thickness of the cortical ribbon in each region. We then determined the fMRI activation magnitude for each region of interest as subjects performed a memory task requiring them to learn and recall word lists given a word-stem cue.
Results: FMRI activation in bilateral CA2,3 and dentate gyrus were significantly correlated with the task paradigm during recall blocks in all subjects; in CA1 it was significantly greater among older adults compared with younger (p=4.3x10-5). Further, fMRI signal change in left CA1 during recall was significantly correlated with posttest score in older subjects (p=.02), but not in younger subjects. Structural measures of cortical thickness found age-related decreases in cortical thickness in the CA1 region and to a lesser extent in ERC. Using multiple regression, we found that thinner entorhinal cortex was more closely associated with memory performance score in older adults compared with younger adults (p=.016). In order to ensure that this relationship was not explained by having more older subjects with thinner entorhinal cortex, we additionally examined the relationship between task score and entorhinal cortex thickness only in older subjects whose entorhinal cortex was in the same range as that of the younger subjects, and found that the relationship was still significant (p=.03).
Discussion: In healthy older subjects, increasing age was associated with increased memory-related blood flow changes on fMRI in the CA1, especially during retrieval, compared to younger subjects. The fMRI changes were not associated with cortical thickness in the same region; rather, age-related thinning of the entorhinal cortex independently predicted verbal memory performance. Together the results are consistent with a model positing increased fMRI activation in intact portions of the hippocampus increase in conjunction with tissue loss in entorhinal cortex; such compensation in normal elderly is associated with better memory performance. These metrics may be useful as surrogate markes for brain changes in subjects at-risk for Alzheimer's Disease.

Longitudinal CSF Isoprostane Measures Predict MCI Transition to AD and Medial Temporal Lobe Atrophy

Mony J. De Leon*, Lisa Mosconi, Susan De Santi, Wai Tsui, Elizabeth Pirraglia, Juan Li, Ken Rich, Elizabeth Javier, Miroslaw Brys, Lidia Sobanska, L.A. Saint-Louis, Anders Wallin, Kaj Blennow and Domenico Pratico

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Background: Very little data exists to evaluate the benefit of longitudinally collecting CSF biological markers for Alzheimer's disease (AD). Moreover, there is virtually no information on the longitudinal relationships between CSF and MRI measures of AD progression. Among the few longitudinal CSF studies published, there is a growing awareness that CSF assays for T-tau or Aβ42 levels while useful in discriminating AD and MCI patients from control, may not be sensitive to disease progression, and thus of limited use in monitoring the course and treatment of disease. On the other hand, prior studies have suggested that CSF isoprostane (IsoP), a marker for oxidative stress, and MRI medial temporal lobe atrophy measures provide evidence of disease progression. We provide evidence of longitudinal IsoP changes in the transition from MCI to AD that are correlated with progressive medial temporal lobe atrophy.
Method: We conducted a longitudinal CSF IsoP and MRI gray matter volume study to examine the prediction of the transition from MCI to AD. 11 normal elderly (NL) volunteers and 48 MCI patients were studied for up to four years. Clinical examinations were conducted that included: medical (history and laboratories), psychiatric, neurological, lumbar puncture, neuropsychological, and MRI. IsoP was assayed by negative ion chemical ionization gas chromatography / mass spectrometry. T1 weighted high-resolution MRI scans were studied using SPM to evaluate the gray matter concentration (GMC). We examined the hypothesis that the baseline and longitudinal IsoP levels would: 1) predict decline to AD and 2) increment the MRI diagnostic accuracy and 3) be associated with progressive medial temporal lobe gray matter loss.
Results: By study end, 26 MCI patients declined to AD and 2 NL subjects declined to MCI (the decline group). At baseline isoprostane (IsoP) levels were elevated 33% in the MCI group as compared to NL. The baseline IsoP measures correctly classified 78% of the declining and non-declining groups [χ 2(1)=18.3, p<.001]. Declining patients had greater rates of IsoP increases (17 %/year) as compared to non-declining MCI (6 %/year). The rate of change in IsoP levels classified the outcome groups with 74% overall accuracy [χ 2(1)=9.8, p<.01]. For 9NL and 8 MCI patients with longitudinal MRI, at baseline, two clusters of reduced GMC were found, these included the left medial temporal lobe ([MTL], (hippocampus, entorhinal cortex (EC) and amygdala) and the precuneus bilaterally. The baseline MTL GMC correctly classified 91% of the outcome groups (χ 2(1)=9.9, p=.002), and the precuneus 74%. The annual rate of MTL GMC change correctly classified 88% of the outcome groups (χ 2 (1)=12.9, p<.001). The rate of change in IsoP levels classified the outcome groups with 94% overall accuracy [χ 2(1)=13.0, p<.001]. Longitudinally, the IsoP measures significantly increased the outcome group classification of the MTL GMC from 88% to 100%. The longitudinal increases in IsoP levels correlated with longitudinal GMC reductions in the MTL (r= -.51).
Discussion: The results from these studies demonstrate that CSF IsoP predicts the transition between MCI and AD, increments the value of quantitative MRI measurements, and demonstrates strong longitudinal effects suitable for monitoring the course of AD and treatment interventions.

Are We Ready for Preventative Trials in People "at Risk" for Dementia?

Trey Sunderland*, Nadeem Mirza, Irene Dustin, Karen T. Putnam, James A. Levy and Robert M. Cohen

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Background: There has been speculation, based on retrospective epidemiologic studies, that both cholesterol-lowering and non-steroidal

anti-inflammatory drugs have a protective effect on Alzheimer's disease (AD) and are associated with a delay in dementia onset. Cerebrospinal fluid (CSF) levels of β-amyloid1-40, 42 are routinely reduced in AD patients compared to controls, and CSF tau and ptau levels are routinely elevated. We hypothsized that even short-term use of statins and NSAIDs in older normal controls "at risk" for dementia might have a significant effect on these important biomarkers.
Methods: Twenty-six healthy older control subjects (11 males, 15 females, mean age=59.8 ± 7.6 years) were treated in a double blind, randomized fashion for 3 months with either lovastatin (40 mg orally QD) or ibuprofen (200 mg orally QID). Subjects underwent lumbar punctures (LPs) for CSF collection at baseline and at the end of the 3-month medication trial, and CSF peptide measures were compared before and after treatment in both groups.
Results: We present evidence that CSF levels of β-amyloid1-42 and β-amyloid1-40 was increased significantly in a group of 26 normal controls after even short-term treatment with either lovastatin or ibuprofen ($p<0.001$) while total tau and ptau231 levels were not significantly changed. There was a significant relationship between the change in serum LDL and the CSF β-amyloid1-42 changes in the Statin subgroup alone ($r=0.60$, $p=0.03$).
Conclusions: While preliminary, these CSF findings are consistent with the speculative hypothesis that Statins, and possibly NSAIDs, may offer a protective effect on the onset of AD via an undetermined β-amyloid mechanism. The inverse correlation between serum LDL and CSF β-amyloid1-42 in the Statin group may also suggest a rationale for higher doses of Statin to enhance the protective benefits for cardiovascular, cerebrovascular and Alzheimer's disease. More importantly, perhaps, the study invites further exploration with biomarker paradigms once more pathophysiologically-relevant medications are available for AD prevention trials.

Panel Session
Genes and Convergent Molecular Pathways in Schizophrenia Pathogenesis

Dysbindin, MUTED, and BLOC1S2: Multiple Susceptibility Genes in the BLOC-1 Complex Implicates Vesicular Trafficking Defects in Schizophrenia

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Background: Numerous studies have established dysbindin (DTNBP1) as an important susceptibility gene for schizophrenia (1), thus functionally linked genes and pathways become primary targets of inquiry. The specific cellular functions of dysbindin are not known, nor are the consequences of the reduced dysbindin transcript (2) and protein levels (3) in postmortem schizophrenic brains. What is known is that dysbindin is a component of the ubiquitously expressed cytosolic BLOC-1 (biogenesis of lysosome-related organelles) complex which includes at least 7 other proteins from the genes MUTED, PLDN, SNAPAP, GCN5L1, BLOC1S2, BLOC1S3, and CNO. Defects in some of these proteins affect the biogenesis and/or trafficking of intracellular organelles found in specialized secretory cells such as pigment cells (i.e. melanocytes and pigment epithelial cells), platelets, T cells and neutrophils. The role of presynaptic neuronal BLOC-1 is unclear, but interesting clues are emerging. Numakawa (4) showed that dysbindin over-expression increased levels of SNAP25 and synapsin-1, elevated phosphorylation of AKT1 (which is itself a dopamine D2 receptor-regulated schizophrenia susceptibility gene), and increased glutamate release, while siRNA knockdown of dysbindin produced opposite effects. PLDN (5) binds to syntaxin 13, a SNARE protein mediating vesicle docking and fusion. SNAPAP may also function with SNAREs via interaction with SNAP25, and overexpression in cultured hippocampal neurons decreased the number of primary dendrites (6).
Methods: We sequenced exons, splice sites and upstream regions of these 8 genes, and genotyped. Family-based and case-control tests of association with the clinical and intermediate phenotypes from the CBDB/NIMH Sibling Study were performed, and statistical epistasis between genes was examined. We transfected lymphoblasts from patients and controls as well as HEK cells to overexpress or knockdown dysbindin and MUTED, and then measured proliferation rate and levels of a number of cell surface proteins, as assayed by FACS.
Results: We found very few coding SNPs, so the impact of risk haplotypes is probably mediated primarily by aberrant levels and timing of expression rather than amino acid substitutions. There was evidence for association between the clinical phenotype and three of the eight genes - dysbindin, MUTED (7) and BLOC1S2. Epistasis between dysbindin and MUTED was evident, and each was in epistasis with COMT, an important determinant of cortical dopamine levels. Functional imaging showed that dysbindin and MUTED influenced activation of PFC and hippocampus during encoding and retrieval and N-back working memory tasks and that MUTED affected hippocampal volume. Surprisingly, only dysbindin was associated with the cognitive phenotypes. We knocked down dysbindin and MUTED by siRNA and found that either reduction was sufficient to decrease cell proliferation and increase the number of dopamine D2, D3 and D5 receptors, while two other proteins (CD11a, CD19) were unchanged.
Discussion: We have only scratched the surface of the cellular and physiologic consequences of allelic variation in the BLOC-1 genes and changes in their protein levels, and may have already observed some relevant connections to other pathways involved in schizophrenia. Dysbindin strongly influenced a broad range of cognitive phenotypes, especially IQ / processing speed, but MUTED (which binds to dysbindin) and BLOC1S2 did not, suggesting that dysbindin may have cellular functions and effects on cognition independent of its role in BLOC-1. References: 1)Straub AJHG 2002 2)Weickert ArchGenPsych 2004 3)Talbot J.Clin.Invest 2004 4)Numakawa HMG 2005 5)Moriyama Traffic 2004 6)Chen MBC 2005 7)Straub AJMG 2005

Towards an Understanding of the Role of DISC1 in Schizophrenia-the Importance of PDE4

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DISC1 is a schizophrenia risk gene with increasing genetic and biological data supporting the plausibility that it may be a major player in the disease. DISC1 was originally identified as the disrupted gene on chromosome 1 due to a balanced translocation t(1;11) which co-segregated with major psychiatric disease including schizophrenia and major depression in a Scottish family. In similar clinical studies a second family with schizophrenia was identified with a t(1; 16) translocation resulting in the disruption of PDE4B on chromosome 1. Intruigingly we identified that DISC1 and PDE4 interact physically through our studies of the DISC1 interactome leading to us characterizing in detail the interaction between DISC1 and PDE4. The UCR2 domain provides a common site through which PDE4 isoforms bind to DISC1. DISC1 and PDE4 interact in vivo and associate in a mitochondrial sub-compartment. DISC1 maintains PDE4 in a 'low-activity' state. The complex is dynamic in nature though as shown by elevated cAMP levels resulting in disassociation of the complex and an increase in the fraction of active, phosphorylated PDE4 present. In unpublished studies we conducted exhaustive yeast-2 hybrid and co-immunoprecipitation/mass spec studies to identify novel DISC1 interactors and thus to gain further insight into DISC1 function. We will, for the first time, present these unpublished data and our hypothesis of a 'DISC1 interactome' where we show that DISC1 is able to bind to a wide range of different molecules. This novel work implicates DISC1 in a wide range of processes which are seen to be pathologically altered in schizophrenia.

Alterations in Neuregulin1 (NRG1)/ErbB4 Signaling Are Related to Specific Intracellular Signaling Pathways in Schizophrenia

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Background: The NRG1/ErbB4 signaling pathway is known to play a critical role in neurodevelopment and adult brain function through its effects on neuronal migration, axonal guidance, synapse formation and the regulation of neurotransmitter function. These events are mediated via intracellular signaling pathways, including the ERK/MAPK and PI3K, and the downstream regulation of the actin cytoskeleton. Genetic association has identified NRG1 and ErbB4 as potential susceptibility genes for schizophrenia and we have previously shown that a molecular mechanism behind the association of NRG1 with schizophrenia involves altered transcriptional regulation of the gene (1). At present, little is known about NRG1 receptors and whether genetic risk via NRG1 or ErbB4 results in perturbation of a specific molecular pathway that mediates NRG1 function in the brain. Using postmortem genomics we have examined ErbB4 and other interacting molecules in the NRG1/ErbB4 signaling cascade. We present evidence that alteration in the splicing of the ErbB4 gene occurs in the brain in schizophrenia and is related to genetic risk for the disease. We propose that altered ErbB4 signaling may represent an upstream effector of intracellular pathways that impact on cellular events related to the control of actin dynamics, brain development and the pathophysiology of the disease.
Methods: We determined gene expression levels for ErbB4 splice isoforms (JM-a, JM-b, CYT-1 and CYT-2) in the hippocampus and dorsolateral prefrontal cortex (DLPFC) of 84 controls and 48 patients with schizophrenia. We examined 3 SNPs in the ErbB4 gene (rs7598440, rs707284, Rs839523), previously reported to constitute a schizophrenia risk haplotype (2). CYT-1 and CYT-2 isoforms of ErbB4 are linked to different intracellular signaling pathways, therefore our investigations were extended to examine a number of molecules that represent downstream targets of ErbB4 activation including, Rac1, AKT1, Cofilin, Slingshot and LIMK1.
Results: Splice variant specific increases in JM-a and CYT-1 ErbB4 expression were observed in the DLFPC in schizophrenia. In the hippocampus, interaction of genotype at all 3 risk SNPs was specifically associated with elevated CYT-1 isoform levels. CYT-1 is linked uniquely to the PI3K pathway, suggesting that there may be a potential bias toward signaling via this cascade in schizophrenia. Data on signal transducer molecules and regulators of the actin cytoskeleton downstream of this pathway will be presented.
Discussion: Our findings suggest that analogous to NRG1, dysregulation of ErbB4 isoform expression may underlie the involvement of the gene in risk for schizophrenia. The functional consequences of altered NRG1/ErbB4 signaling on cortical neural development, plasticity and neurotransmission will be discussed in the context of aberrant regulation of intracellular signaling cascades linked to the organization of the actin cytoskeleton. 1) Law et al, 2006. Neuregulin 1 (NRG1) Transcripts are differentially expressed in schizophrenia and regulated by 5' snpS associated with the disease. PNAS in press. 2) Silsberg et al, 2006. Am J Med Genet B Neuropsychiatr Genet. 5;141(2):142-8.

BDNF Synthesis and Processing Pathways Relevant to Cellular Mechanisms of Schizophrenia Pathogenesis

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Brain derived neurotrophic factor (BDNF), a member of the neurotrophin family of secreted neurotrophic factors in the brain, has recently emerged as a key regulator of synaptic plasticity and cognitive brain functions. Numerous studies have reported association of schizophrenia with variations in BDNF gene or changes in the levels of BDNF mRNA and protein in the brain. However, the specific links between BDNF and schizophrenia remain to be established. Multiple molecular pathways (DISC, dysbindin, neuregulin) discussed in this panel may affect cortical microcircuit through common synaptic mechanisms such as long-term potentiation (LTP). As a critical factor for synaptic plasticity, BDNF may also alter the capacity of these pathways to impact on cognitive functions. For example, BDNF controls the expression, secretion and signaling of neuregulin, another secretory factor involved in LTP. Recent studies in the cell biology of BDNF have provided new insights into how BDNF signaling could impact the molecular etiology of schizophrenia. The val-met polymorphism in the pro-domain of BDNF affects activity-dependent secretion of the protein, leading to changes early phase LTP (E-LTP) and short-term memory. The C270T polymorphism in the 5'UTR of BDNF mRNA may impact dendritic mRNA trafficking or translation, resulting in alterations in neuronal excitability and synaptic transmission. Environmental factors, such as stress or fear, antipsychotic drugs, and age also influence BDNF expression. The expression of BDNF gene is controlled by multiple promoters, each drives expression of BDNF transcripts in different brain regions, cell types, and subcellular compartments (dendrites, cell body, etc), and each is regulated by different genetic and environmental factors. Mice lacing promoter III-driven BDNF transcription exhibit specific impairments in late phase LTP (L-LTP) and long-term memory, but not E-LTP and short-term memories. Moreover, BDNF is initially synthesized as a precursor (proBDNF), which is proteolytically cleaved to form mature BDNF (mBDNF). It is now recognized that proBDNF and mBDNF, by interacting with p75NTR and TrkB receptors respectively, could elicit opposite effects on plasticity (LTD and LTP, respectively). Thus, identification of changes in specific BDNF transcripts and specific BDNF isoforms associated with schizophrenia will significantly improve our understanding of how BDNF signaling contributes to cellular mechanisms of schizophrenia pathogenesis. Moreover, alteration of the molecular pathways may lead to pathological changes in schizophrenia by impacting on a common intermediate step-synaptic plasticity, which is critically regulated by BDNF.

Panel Session
Intracellular Mechanisms for Regulating Cell Sensitivity to Corticotropin-Releasing Factor and Stress

The Regulation of CRF2 Expression: Implications for Stress-Related Psychopathology

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Background: The CRF system has two receptors, CRF1 and CRF2, which are implicated in mediating stress-related psychopathology. While CRF1 has been well studied, considerabaly less is known about the regulaton and function of CRF2. It is of interest that compared to rodents,primates have a much wider brain distribution of CRF2. We focused on CRF2 for the above reasons as well as its potential importance in mediating psychopathology.
Methods: Receptor autoradiography and in situ hybridization techniques were used to characterize the distribution of CRF2 in human brain. Molecular techniques were used to clone the promoter region of the human CRF2 and studies were performed to assess regulatory mechanisms. Finally, site specific administration of a CRF2 antagonist was performed to understand the role of this receptor in mediating anxiety.
Results: Our studies of the distribution of CRF2 in human amygdala and temporal cortex demonstrated high levels of expression in etorhinal cortex compared to amygdala. This distrubtion of CRF receptors differs from that of rodents suggesting a more prominent role for CRF2 in mediating anxiety in primate species. To further elucidate the function of CRF2, we clonled the promoter region of the receptor and identified functional regulatory elements that are responsive to glucocorticoids and cAMP. These findings point to possible

mechanisms through which stress can alter the activity of the CRF system. To understand the functional role of CRF2, we performed studies administering a CRF2 antagonist into the lateral septum of rats exposed to stress. The results demonstrate that CRF2 activation mediates shock-induced freezing behavior.
Discussion: Taken together, these results suggest mechanisms that regulate CRF2 expression. Furthermore, the studies demonstrate a role for CRF2 in mediating anxiety and suggest that overactive CRF2 systems may mediate the abnormal expression of fear responses that can become dysregulated in stress-related disorders such as anxiety and depression.

Agonist-Induced Internalization of Corticotropin-Releasing Factor Receptors in Noradrenergic Neurons of the Rat Locus Coeruleus
Elisabeth Van Bockstaele* and Rita Valentino

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Background: Agonist-induced trafficking of receptors plays a significant role in modulating cellular sensitivity to neurotransmitters. Agonist stimulation of G-protein coupled receptors can result in internalization of the receptor protein into intracellular vesicles. Subsequently, receptors are recycled to the cell surface or further transported to lysosomes for eventual degradation. Corticotropin releasing factor (CRF), the hypothalamic neurohormone that initiates the endocrine limb of the stress response, acts as a neurotransmitter that directly mediates locus coeruleus (LC) activation by stressors. Internalization of CRFr, a G-protein coupled receptor, is one potential cellular mechanism for regulating postsynaptic sensitivity to CRF. Although there is evidence for CRFr internalization in cultured neurons, this has not been demonstrated in vivo. Cellular trafficking of the CRFr within LC neurons was examined after CRF or vehicle injection into the LC.
Methods: Rats were anesthetized with halothane and surgically prepared for LC recording and local microinjection using double barrel micropipettes. When stable, unitary action potentials were isolated, intracoerulear infusions were made by applying small pulses of pressure to the peptide-containing barrel. Artificial cerebrospinal fluid (ACSF; 100 nl) or ovine CRF (100 ng in 100 nl ACSF) was microinjected into the LC and LC discharge was recorded. Rats were perfused 5 (n=3 or 4 for ACSF and CRF, respectively) or 30 min (n=4 or 5 for ACSF and CRF, respectively) later. For electron microscopy (EM), tissue was obtained from 16 rats that were injected with either ACSF or CRF into the LC. CRFr immunoreactivity was visualized using immunogold-silver labeling. Intensification of the silver grains was achieved using a silver enhancement kit. TH immunoreactivity was detected using biotinylated donkey anti-mouse IgG and avidin-biotin complex solution. For quantification, the measure of internalization was the ratio of cytoplasmic to total silver grains in a dendrite. Silver grains were identified as plasmalemmal if they were associated with the plasma membrane, and cytoplasmic if they were not in contact with the plasma membrane. This ratio was determined for each dendrite and the mean determined per animal.
Results: Immunogold-silver labeling for CRFr was localized to the plasma membrane of tyrosine hydroxylase (TH)-immunoperoxidase labeled dendrites. CRF (100 ng) injection into the LC produced a robust neuronal activation that peaked 10-15 min after injection and was maintained for the duration of the recording. This was associated with CRFr internalization in LC neurons that was apparent at 5 and 30 min after injection. By 5 min after injection the ratio of cytoplasmic:total dendritic CRFr-labeling was 0.81±0.01 in rats injected with CRF and 0.59±0.02 in rats injected with artificial cerebrospinal fluid (ACSF; $P < 0.0001$). Enhanced internalization of CRFr was maintained at 30 min after CRF injection, with the ratio being 0.86±0.02 for CRF-injected cases and 0.57±0.03 for ACSF-injected cases ($P < 0.0001$). Internalized CRFr was associated with early endosomes, indicative of degradation or recycling.
Discussion: Using ultrastructural analysis, we provide the first in vivo evidence for agonist-induced internalization of CRF receptor (CRFr) in rat LC. Agonist-induced CRFr internalization in LC neurons may underlie acute desensitization to CRF or stress. This process may be a pivotal target by which stressors or pharmacological agents regulate the sensitivity of the LC-NE system to CRF and subsequent stressors.

Differential Mechanisms of Agonist and Antagonist Regulation of Conformation and Function of the CRF_1 Receptor
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Stress and stress-related disorders have long been associated with a dysregulation of neurotransmitter systems in the central nervous system and corticotropin-releasing factor (CRF) is thought to be the principle physiological regulator of an organism's response to stress. Within the last two decades, the CRF system has matured into a complex family of multiple receptor subtypes with multiple endogenous ligands ranging in scope of effects from centrally mediated mood disorders to cardiovascular effects to the control and manifestation of gut disorders. CRF receptors exist as two subtypes (CRF_1 and CRF_2) with the CRF_2 receptor existing as three independent isoforms ($CRF_{2(a)}$, $CRF_{2(b)}$ and $CRF_{2(c)}$), each discretely localized within the body. The family of ligands associated with this system contains CRF itself, the endogenous functional ligand for the CRF_1 receptor, and the subfamily of the urocortins (UCN1, UCN2 and UCN3). UCN2 and UCN3 are specific for the CRF_2 receptor and thought to be the primary mediators of CRF_2 receptor function, although UCN3 has lower affinity. UCN1 has equal affinity for both receptor subtypes and is localized in areas where it can modulate (centrally and peripherally) the activity of both receptors. In addition to the peptide ligands, the discovery of small molecule, orally active non-peptide antagonists has served to elucidate differences in the regulation of these receptors. While detailed structure-activity relationship (SAR) analysis of peptide and nonpeptide ligands has given us tremendous insight into the structural requirements of the receptor, very little is currently known of the discrete molecular mechanisms involved in these interactions. In the present study, we provide direct evidence for the differential binding, activation and regulation of the CRF_1 receptor by peptide agonists as well as peptide and non-peptide receptor antagonists. CRF and other agonist peptides bind first to the N-terminal domain with moderate affinity (50 nM) and then to the transmembrane domain (TM), generating first a high affinity interaction (0.2 nM)and then an active conformation of the receptor capable of functional signaling. Peptide antagonists such as astressin bind with high affinity (0.15 nM) to the N-terminal domain but due to the truncated nature of their N-termini, do not strongly bind to and interact with the TM domain. Peptide antagonists therefore block agonist function through orthosteric overlapping binding sites with the agonist peptides at the N-terminal domain. The non-peptide compounds on the other hand, bind with high affinity well within the TM domain and act allosterically to inhibit the agonist-induced conformational change of the receptor, and this interaction is independent of the functional state (G-protein coupled or uncoupled) of the receptor. These findings provide evidence for non-overlapping sites of action between peptides and non-peptides and imply that non-peptide antagonists act in an allosteric manner to inhibit the function of the endogenous peptides. We also provide evidence that both peptide agonists (CRF) and antagonists (astressin) cause internalization of CRF_1 receptors, but only CRF produces a frank down-regulation or desensitization. Importantly, this property is not shared by non-peptide antagonists. Taken together, these data suggest that treatment with peptides, albeit for peripheral indications, would have markedly different effects on the CRF system than non-peptide molecules. Non-peptide antagonists for the CRF_1 receptor seem to be neutral inhibitors of function most likely having no effect on receptor regulation, thus reducing their potential of direct receptor-mediated liabilities in the treatment of neuropsychiatric and stress-related disorders.

Effects of Corticotropin-Releasing Factor and Related Peptides on Dendritogenesis of Brain Monoamine Neurons: Role of Rho GTPase Regulators of the Actin Cytoskeleton

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Cognitive aspects of the acute stress response are partly mediated through activation of the locus coeruleus (LC)-norepinephrine (NE) system via corticotropin-releasing factor (CRF). CRF increases LC neuronal activity in vivo and in vitro through actions at CRF1 receptors. This is necessary for forebrain electroencephalographic activation (an indicator of arousal) by certain stressors. In addition to acutely affecting neuronal activity, CRF can have a long term impact on brain function by altering neuronal structure. This has been demonstrated in hippocampus and cerebellum. The present study examined the effects of CRF on morphology of LC-NE neurons in vitro. LC slice cultures from newborn rats were exposed to CRF for 12 h to 7 days. CRF increased dendritogenesis of LC-NE neurons, as indicated by the number of dendrites ($p<0.05$), dendritic branching ($p<0.001$) and length of longest dendrite ($p<0.001$). This effect was sensitive to antagonism by astressin, but not by the CRF2 antagonist, antisauvagine-30. CRF activated protein kinase A (PKA) and protein kinase C (PKC) and increased levels of phosphorylated mitogen-activated protein kinase (MAPK) in these preparations and these effects were prevented by astressin. Incubation of slices (5 days) with antagonists of PKA and MAPK, but not of PKC, prevented the effect of CRF on dendritic length, implicating these particular pathways in the structural effect. Because members of the Rho family of GTPases regulate the dynamics of the actin and microtubule cytoskeleton, the role of these mediators in the structural effects of CRF were examined. Incubation with CRF increased levels of phosphorylated Rac1 and RhoA in the slice cultures. The effects of CRF on dendritic growth of LC neurons could be attributed to activation of Rac1 because CRF effects on dendritic length were prevented in cultures that were incubated with NSC23766, a compound that interferes with the action of Rac1. In contrast, Y27632, which interferes with the function of Rho A, did not alter the effect produced by CRF. Thus, the Rho GTPase, Rac1, may serve to link intracellular signaling engaged by CRF1 activation directly to actin cytoskeleton dynamics. The overall effect of CRF in LC slices, i.e., the production of a dendritic field that extends beyond the cluster of LC neurons, has important functional implications. The pericoerulear region (peri-LC) into which LC dendrites extend is targeted by axon terminals from regions that are distinct from those targeting the nuclear core of the LC. Particularly, axon terminals from the central nucleus of the amygdala (CNA) and bed nucleus of the stria terminalis terminate in the pericoerulear region where they form synaptic contacts with LC dendrites. Thus, this is an important site at which the limbic system communicates with the LC. By governing dendritic extension into the peri-LC, CRF may determine the degree to which the LC-NE system is regulated by limbic structures that are involved in emotional expression and thereby, the magnitude of emotional arousal. We speculate that by promoting LC dendritic extension, chronic or repeated exposure to CRF can increase the probability for LC dendritic contacts with limbic afferents, thereby facilitating the formation of circuits that underlie emotional arousal and anxiety. Supported by PHS Grant MH40008.

Panel Session
Mechanisms of Substance Abuse Risk in ADHD

Modeling Impulsivity and Substance Abuse Disorders in Rats: New Insights Using Positron Emission Tomography

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Background: A number of studies indicate elevated frequencies of drug abuse among individuals who are also diagnosed with antisocial personality disorder, depression, and attention deficit/hyperactivity disorder (ADHD). However, co-morbid disorders such as these could manifest as a consequence of chronic drug exposure or be present before the onset of drug-taking behavior. Resolving this issue is difficult in human drug abusers because the nature of the drug exposure cannot easily be controlled. This presentation presents new findings on an animal model of trait impulsivity based on a spontaneously occurring impulsive phenotype in outbred Lister hooded (LH) rats (<7% incidence), and which is associated with abnormally high rates of intravenous cocaine self-administration. It considers, in particular, the relationship between trait impulsivity and cocaine reinforcement, and the underlying neural substrates mediating these behavioral processes.
Methods: Male LH rats were screened for trait impulsivity on a task that shares similarities with the continuous performance test of sustained visual attention in humans (the so-called '5-choice serial reaction time task'), specifically as measured by an inability to refrain from inappropriate responding prior to the presentation of a visual target stimulus. Micro-positron emission tomography (PET) was used to investigate D2/D3 receptor function in the striatum of drug-naive, halothane-anaesthetised, trait high (n=7) and trait low (n=7) impulsive rats using the selective high affinity PET ligand [18F]-fallypride. The same subjects were subsequently trained to self-administer cocaine by the intravenous route (0.25 mg/infusion) for extended periods (five daily 8h sessions or 'cycles' repeated every 9 days for 5 cycles) under a continuous schedule of reinforcement (FR1).
Results: In addition to deficits in impulse control on the 5-choice task, trait impulsive rats also showed attentional disturbances with a significantly increased number of incorrect choice responses. Micro-PET findings indicate reduced binding potentials of [18F]-fallypride in the dorsal striatum of trait impulsive rats compared with the control group (reference tissue model, cerebellum), especially the anterior dorsomedial striatum (maximum reduction 24%). Trait impulsive rats also exhibited an escalation of intravenous cocaine self-administration (mean rate: 35 infusions/hour) compared with trait low impulsive rats (mean rate: 22 infusions/hour). Finally, cocaine withdrawal was associated with a selective reduction in impulsivity in the trait impulsive group, which returned to pre-cocaine levels following a protracted 3-month period of withdrawal.
Discussion: These findings provide new insights into the inter-relationship between pre-morbid impulsivity and addictive-like behaviour in the rat. In particular, these data provide evidence for a pre-disposition to self-administer cocaine based on trait impairments in impulse control, and thus favour the hypothesis that impulsivity may be an important contributory factor rather than a consequence of chronic substance abuse. These findings are also relevant to analogous imaging studies in human cocaine addicts (e.g, ND Volkow and colleagues), specifically by demonstrating that pre-existing abnormalities in striatal D2 receptors may be importantly linked to impulsivity and substance abuse liability. Supported by the MRC (UK) and Wellcome Trust within the University of Cambridge Behavioural and Clinical Neuroscience Institute.

Behavioral and Neural Substrates of Decision-Making Processes in Adolescents as Potential Markers of Risk for Substance Use

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Adolescence is a period of heightened risk for substance use initiation and transition to substance abuse. ADHD, the psychiatric disorder of the highest prevalence in youth, potentiates this risk. Symptoms, rather than disorder per se, seem to contribute to the liability for substance use. Impulsivity and aggression are among the most frequently noted behavioral associations with vulnerability for

addiction. Unique patterns of responses to incentives (reward and punishment) may also contribute to the risk. Data will be presented for both behavioral and neural factors that are likely to confer risk for substance use. From a behavioral perspective, we recently completed a 5-year longitudinal study of healthy and ADHD adolescents that impulsivity and aggression were uniquely associated with initiation of alcohol and tobacco/marijuana use respectively. Using a well-validated gambling task, we also showed that healthy adolescents were more risk-seeking than healthy adults, and that adolescents with ADHD were more risk-taking than healthy adolescents. From a neural perspective, using fMRI paired with decision-making paradigms, we recently showed that, in response to reward, healthy adolescents engaged the ventral striatum more strongly than healthy adults, and healthy adults engaged the amygdala and prefrontal cortex more strongly than healthy adolescents, suggesting a developmental change in the balance of the contribution of the neural circuits associated with approach behavior and those associated with avoidance behavior and cognitive control in goal-directed behavior. The distinct function of the ventral striatum in adolescence was also found in an fMRI study by Bjork et al. 2004. Finally, using the same task as Bjork et al., we found, very recently, in a temperament study of children characterized since infancy, differences between exuberant adolescents and normally regulated adolescents that were in the direction of Bjork et al.'s findings, suggesting that exuberant adolescents could stand as "super-adolescents". Although none of these exuberant adolescents had a diagnosis of ADHD, it is conceivable that a subset of adolescents with ADHD would present similar temperamental characteristics as these exuberant adolescents. Finally, the same pattern of ventral striatum activity was found in a cerebral blood flow PET study of adults with ADHD who were performing the gambling task already mentioned above. Together, these findings, most of them from very recent work (2005, in press, and under review), suggest specific functioning of the reward system in adolescents with ADHD, that could represent a risk factor for substance use and addiction. Based on the common involvement of dopamine function in ADHD, substance use and reward function, future studies are needed to better isolate the neurobiological substrates that underlie risk, combining reward-related paradigms with pharmacological probes, from a developmental perspective.

Development of Substance Use Among Youth with ADHD

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Background: The Multimodal Treatment Study of ADHD (MTA) randomly assigned 579 7.0-9.9 year old children with ADHD at six sites to medication management, behavior therapy, the combination of these treatments, or community care. The initial results of this 14-month treatment trial have been described in widely read papers (e.g., MTA Cooperative Group, 1999a; 1999b; Swanson et al., 2001; Conners et al., 2001), and the set of papers describing the 3-year outcomes are undergoing post-review revisions. Because of the young age of the sample, it was only by the 2- and 3-year follow-up assessments that study of emerging substance use was feasible. These results, also presented at the American Academy of Child and Adolescent Psychiatry in 2005, showed higher rates of substance use initiation among the MTA children than among comparison youth selected for their demographic similarity (local normative comparison group, or LNCG), 17.4% MTA vs. 7.8% LNCG. Effects of medication (study-assigned or later self-selected) were not found. Instead, youth who received study-assigned behavior therapy were less likely to report substance use at the two-year follow-up. These effects dissipated one year later (Molina et al., under review) as had treatment effects on the other primary outcomes (MTA Cooperative Group, under review).
Methods: The MTA children are still being followed, and data describing their functioning at two assessments in the 13-18 year old age range are currently being analyzed. These data will be important for describing the progression of substance use in a large well-characterized multi-site sample as children mature through a high risk period. Developmentally appropriate measures of substance use will be particularly informative (Molina & Pelham, 2003).
Results: Preliminary findings at the 6-year follow-up (mostly 14-16 years old) indicate more daily cigarette use by age 16 (15.6% MTA vs. 2.4% LNCG, p=.01) and more repeated marijuana use (19.2% MTA vs. 9.9% LNCG, p=.004). Rates of alcohol use do not appear to differ and rates of other illicit drug use are low. These analyses will be extended to the 8-year follow-up when the youth are roughly 16-18 years old; abuse/dependence will also be reported. We predict no residual effects of randomized treatment; analyses will test associations with naturally selected medication treatment in adolescence, a controversial and hotly debated area of inquiry.
Discussion: Investigators Molina and Pelham have also been simultaneously following a large sample of children with ADHD (n=364) and non-ADHD comparison youth (n=240) from Pelham's ADD Program in Pittsburgh, aka the Pittsburgh ADHD Longitudinal Study. In contrast to the MTA, this study has as its focus the onset, course, and development of alcohol and other drug use. Results from this study will be used to describe developmental issues in the study of substance use among these participants, to further illustrate the elevated risk for substance use among youth with childhood ADHD, and to discuss mediators and moderators of ADHD risk (e.g., cognitive processes including coping skills, expectations for the positive and negative effects of alcohol, gravitation toward deviant peers, onset of delinquent behavior, and parental influences). Together the results of these two studies, and overview of existing research, will provide a backdrop for the remaining symposium presentations.

Disrupted Brain Dopamine Activity in ADHD: Comparison with that in Addiction

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ADHD is associated with an increased risk for substance abuse. The mechanisms underlying this vulnerability are not properly understood and though automedication is likely to contribute, common neurobiological substrates may also underlie the comorbidity. Imaging studies have documented abnormal brain dopamine (DA) function in drug addiction (decreases in DA D2 receptors and in DA release in striatum) and imaging studies in ADHD, although not always consistent, also seem to point to an involvement of the brain DA system. Here we investigated the brain DA system in 20 never medicated adults with ADHD and in 24 controls and compare the findings from those we had previously obtained in cocaine addicted subjects. We used positron emission tomography (PET) to measure DA transporters (DAT) (using the DAT radioligand [11C]cocaine), DA D2 receptor availability (using [11C]raclopride) and DA release (difference in the binding of [11C]raclopride after placebo and after 0.5 mg/kg of intravenous methylphenidate, which by blocking DAT allows DA to accumulate in the extracellular space as a function of the rate of its release). DAT measures in ADHD subjects were significantly lower than in controls (C) in left (Bmax/Kd C = 1.88 ±.14 ADHD 1.77 ±.15 $p < 0.02$), and right caudate (Bmax/Kd C = 1.90 ±.14 ADHD 1.81 ±.13 $p < 0.04$) but did not differ in putamen. D2 receptor availability in ADHD was significantly lower than in controls in left caudate (Bmax/Kd C = 2.99 ±.34 ADHD 2.76 ±.38 $p < 0.05$) but did not differ in putamen. Changes in [11C]raclopride binding after MP showed a trend for a blunted response in ADHD subjects than in controls in left caudate ($p < 0.06$). SPM analyses corroborated the reduction in DAT in caudate and the reductions in D2 receptors in left caudate but also documented a significant reduction ($p < 0.005$) both in DAT and D2 receptors in a region where the Nucleus Accumbens and

BA 25 are located. SPM also showed a significantly smaller volume of change in [11C]raclopride binding after iv MP in ADHD subjects than in controls. Though these studies document abnormalities both in markers of presynaptic and postsynaptic DA sites the pattern is different from that seen in cocaine abusers. Specifically in cocaine abusers but not in ADHD we showed reduction in D2 receptors and in DA release in putamen whereas we showed no evidence of striatal DAT changes (as assessed with [11C]cocaine). The extent to which the DA abnormalities in NAc in ADHD could contribute to a vulnerability for substance abuse merits investigation.

Panel Session
Recent Findings from the STAR*D Trial

STAR*D: Design, Rationale, and Recurrent Acute and Longer-Term Results

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The Sequenced Treatment Alternatives to Relieve Depression trial (STAR*D) enrolled over 4,000 outpatients with nonpsychotic major depressive disorder from 18 primary care and 23 specialty care settings in the public and private sectors. Comparisons of the patients from these two settings revealed very few differences in clinical or demographic features at baseline. At every treatment step, treatment procedures employed the routine use, at each clinic visit, of brief measures of symptoms (the Quick Inventory of Depressive Symptoms-QIDS) and side effects (the Frequency, Intensity and Burden of Side Effects-FIBSER). Protocol manuals, didactic training, and a web-based monitoring system provided guidance to ensure tolerable but vigorous dosing at each treatment step. For step 1 (Level 1) citalopram (up to 60 mg/day) was provided to all subjects for up to 14 weeks of treatment. Step 2 (Level 2) entailed up to seven different treatment options which included switching to bupropion-SR, sertraline, venlafaxine-XR, or cognitive therapy (CCT). In addition, three different augmentations to citalopram (CIT) were available (CIT+BUP-SR, CIT+CT, CIT+VEN-XR). Subsequent steps included mirtazapine (MIRT) or nortripyline (NT) as switch medications, or lithium or an MAOI or T3 augmentation (Level 3) and an MAOI (tranylcypromine) vs VEN-XR+MIRT (Level 4). Treatment options were presented in an equipoise stratified randomized design. At the time of presentation, we will be able to report on the response and remission rates that were associated with each of the four acute treatment steps for which participants could enroll. In addition, the long-term follow-up data, from the 1 year naturalistic follow-up (available to all participants with a clinically acceptable benefit with any acute treatment) will be presented.

Remission of Mothers Depression and Psychopathology in the Children: A STAR*D Child Report

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Children of depressed parents have high rates of anxiety, disruptive, and depressive disorders that begin early, often continue into adulthood, and are impairing. The appropriate treatment of these children is not well defined. We determined whether effective treatment of maternal depression was associated with reduction of symptoms and diagnoses in their children. Children whose depressed mothers were being treated with medication as part of the multi-center Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial conducted in broadly representative primary and psychiatric outpatient practices were assessed by a team of evaluators not involved in maternal treatment and unaware of maternal outcomes. 151 mother-child (age 7-17) pairs in 8 primary care and 11 psychiatric outpatient clinics across 7 Regional Centers were studied. The 3 month data, when all mothers were on antidepressant medication, showed that remission of maternal depression was significantly associated with reductions in the children's diagnoses and symptoms. There was an overall 11% decrease in rates of diagnoses in children of remitted mothers as compared to an approximately 8% increase in rates of diagnoses in children of non-remitted mothers. This rate difference remained statistically significant after controlling for the child's age and gender, and possible confounding factors ($p = 0.013$). Of the children with a diagnosis at baseline, remission was reported in 33% of those whose mothers remitted, compared to only a 12% remission rate among children of mothers who did not remit. Of children with no diagnoses at baseline, all children of remitted mothers remained free of psychiatric diagnoses at 3 months, whereas 17% of the children of non-remitted mothers acquired a diagnosis. This paper presents new findings on the child outcomes during one year after initiation of the mother's treatment. We report on the effects on children of maternal relapse and late remission.

Moderators of Response to Next-Step Strategies in Resistant Depression

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There is now good evidence that the majority of patients with major depressive disorder (MDD) do not achieve remission with their first antidepressant trial. The need for next-step interventions is therefore more the rule and not the exception. However, clinicians are than faced with the dilemma of how to choose next-step treatment and it is therefore very important to be able to identify what variables may predict who should be treated with a given next-step treatment? Clinicians have attempted to answer these questions ever since researchers established that antidepressants were superior to placebo in the treatment of depression. The literature is filled with studies examining possible moderators of antidepressant response in treatment-resistant patients, although most of the studies were grossly underpowered and often unreplicated. In the course of the Sequenced Treatment Alternatives to Relieve Depression trial (STAR*D), outpatients with MDD who did not remit or were intolerant to antidepressant treatment with the SSRI citalopram were able to enter one or more subsequent treatment steps (Levels 2, 2A, 3 and 4) for acute treatment. These STAR*D randomized comparisons of next-step strategies for treatment-resistant patients provide important information on the relative effectiveness of these treatments, but also offer an unique opportunity to identify baseline clinical moderators that could have clinical relevance in selecting among the different second and third treatment steps (Level 2). Dr. Fava will summarize the findings from the analyses concerning possible moderators of acute response to treatment during levels 2-4 of STAR*D and will discuss the implication of these findings for future research and current practice.

Pharmacogenetics of Treatment Outcome and Side Effects in the STAR*D Cohort

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Background: Important components of the individual variability in treatment outcome and side effects among patients treated with antidepressants may have a basis in common genetic variation that can be revealed directly through genetic association methods.
Methods: We are genotyping DNA samples obtained from 1953 patients with major depressive disorder who were treated with antide-

pressants in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study and prospectively assessed. So far we have focused on a selection of 68 candidate genes which we have genotyped with 768 single nucleotide polymorphism markers chosen to detect common genetic variation. The goals are 1) to identify genetic markers of treatment outcome and 2) to identify genetic markers of treatment emergent side effects.
Results: So far, we have detected significant and reproducible association between response to citalopram treatment and markers in HTR2A, which encodes the serotonin 2A receptor (McMahon et al 2006), and GRIK4, which encodes a kainate-type glutamate receptor.
Discussion: Taken together with prior neurobiological findings, these genetic association data make a compelling case for key roles of HTR2A and GRIK4 in the mechanism of antidepressant action. Studies of treatment-emergent adverse events, such as insomnia and anxiety, are currently underway. Genes involved in these events may also modulate risk for bipolar or anxiety disorders. Genetic markers that identify individuals at high risk for treatment failure or particular side effects may ultimately help guide treatment decisions.

Panel Session
Role of Cardiovascular Drugs with Anti-Hypertensive Properties in the Treatment of Alzheimer's Disease Dementia

Antihypertensive Medication Use and Incident Alzheimer Disease: The Cache County Study

Ara S. Khachaturian, Peter P. Zandi, Constantine G. Lyketsos, Kathleen M. Hayden, Ingmar Skoog, Maria C. Norton, JoAnn T. Tschanz, Lawrence S. Mayer, Kathleen A. Welsh-Bohmer and John C. Breitner*

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Background: Recent reports suggest that antihypertensive (AH) medications may reduce the risk of dementing illnesses. We examined the relationship of AH medication use with incidence of Alzheimer disease (AD) among the elderly population (aged 65 years and older) of Cache County, Utah, and examined whether the relationship varies with different classes of AH medications.
Methods: After an initial (wave 1) multistage assessment (1995 through 1997) to identify prevalent cases of dementia, we used similar methods 3 years later (wave 2) to identify 104 incident cases of AD among the 3,308 survivors. At the baseline assessment, we obtained a detailed drug inventory from the study participants. We carried out discrete time survival analyses to examine the association between the use of AH medications (including angiotensin converting enzyme inhibitors, beta-blockers, calcium channel blockers, and diuretics) at baseline with subsequent risk of AD.
Results: Use of any AH medication at baseline was associated with lower incidence of AD (adjusted hazard ratio, 0.64; 95% confidence interval, 0.41-0.98). Examination of medication subclasses showed that use of diuretics (adjusted hazard ratio, 0.57; 95% confidence interval, 0.33-0.94), and specifically potassium-sparing diuretics (adjusted hazard ratio, 0.26; 95% confidence interval, 0.08-0.64), was associated with the greatest reduction in risk of AD. Corresponding analysis with a fully examined subsample controlling for blood pressure measurements did not substantially change our findings.
Conclusion: These data suggest that AH medications, and specifically potassium-sparing diuretics, are associated with reduced incidence of AD. Because the latter association is a new finding, it will require confirmation by further study.

Development of Cardiovascular β-Amyloid Lowering Drugs as a Treatment in Alzheimer's Disease

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Background: Recent epidemiological evidence indicates that certain cardiovascular antihypertensive agents decrease AD incidence, though additional investigations have failed to support these findings.
Methods: We hypothesize that this apparent inconsistency may be related to the existence of varying AD disease-modifying activities within this class of antihypertensive agents. Based on this consideration and the fact that brain β-amyloid (Aβ) neuropathology is a major hallmark of AD, we tested the possibility that certain antihypertensive agents affect AD progression, in part by influencing formation of Aβ peptides within the brain. Excitingly, in ongoing high-throughput screening of 150 commercially available cardiovascular drugs, we identified 24 agents capable of significantly promoting Aβ-lowering activities in primary neuron cultures derived from embryonic Tg2576 mice used to model AD.
Results: When these 24 agents were grouped according to similarities in pharmacologic activity, we found that 21 of these drugs also have antihypertensive properties. Most excitingly, our preclinical investigations showed that valsartan (a widely-prescribed antihypertensive drug and one of the above-noted 24 antihypertensive agents with Aβ-lowering activities) also significantly attenuated spatial memory deterioration and decreases Aβ content of the brain in Tg2576 mice in a dose-dependent manner independent of its blood-pressure lowering activities.
Discussion: The studies strongly support new evidence that certain anti-hypertensive drugs may prevent AD clinical progression through prevention of amyloid plaque neuropathology. This information will eventually be used in preclinical development of novel "lead" Aβ-lowering compounds for AD prevention and/or therapeutics.

Effect of Baseline Cognitive Function and Antihypertensive Treatment on Cognitive and Cardiovascular Outcomes: Study on COgnition and Prognosis in the Elderly (SCOPE)

Ingmar Skoog*, Hans Lithell, Lennart Hansson, Dag Elmfeldt, Albert Hofman, Bertil Olofsson, Peter Trankwalder and Alberto Zanchetti

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Background: We examined whether cognitive function at baseline affected cognitive and cardiovascular outcomes in the Study on COgnition and Prognosis in the Elderly (SCOPE), a blood pressure (BP)-lowering intervention trial.
Methods: SCOPE included 4937 patients, aged 70 to 89 years, with mild-to-moderate hypertension and Mini Mental State Examination (MMSE) score > or =24. Double-blind treatment was initiated with candesartan or placebo. Open-label therapy was added as needed to control BP, both in the candesartan (49%) and control (66%) groups. Mean follow-up was 3.7 years. Low cognitive function (LCF) at baseline was defined as MMSE score 24 to 28 (N = 2070), and high cognitive function (HCF) as MMSE score 29 to 30 (N = 2867).
Results: Mean BP reductions were approximately 20/10 mm Hg both in LCF and HCF patients, with greater reductions in the candesartan group than in the control group. The incidence of dementia was higher in LCF than in HCF patients. A higher cardiovascular event rate observed in LCF patients was explained by older age and other cardiovascular risk factors at baseline. In LCF patients, the MMSE score declined less in the candesartan than in the control group (mean difference 0.49, 95% confidence interval 0.02 to 0.97, P = .04). Nonfatal stroke was reduced in the candesartan group in the total sample (28%, P = .04), with no difference between LCF (27%) and HCF (29%) patients.
Conclusions: Elderly patients with mild-to-moderate hypertension and slightly impaired cognitive function (MMSE 24 to 28) are at increased risk of dementia and cardiovascular events. This analysis indicates that effective antihypertensive therapy may reduce cognitive decline and stroke incidence in these patients.

Panel Session
Salvinorin A: From Natural Product to Validated Molecular Target for Mood-Related Disorders

Salvinorin A: History, Discovery and Molecular Pharmacology

Bryan L. Roth*

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In this talk I will present data on the medicinal chemistry, molecular and cellular biology of salvinorin A's actions on KORs. I will also give an overview of salvinorin A and Salvia divinorum. Salvia divinorum is a hallucinogenic plant from the Sage family which has been used for divination and shamanism by curanderos for many centuries in Central America. Salvia divinorum was first reported by Gordon Wasson more than 40 years ago and the presumed active ingredient identified as salvinorin A in the early 1960's by Leander Valdes. It was subsequently discovered that salvinorin A is an extremely potent and rapid-acting hallucinogen (Siebert J Ethnopharmacol. 1994). Salvinorin A and Salvia divinorum have recently gained widespread attention in the media because of it is readily available via the internet (where it is sold as a legal hallucinogen). Salvinorin A and Salvia divinorum are currently non-scheduled by the US Drug Enforcement Agency although two states and several countries have outlawed the sale and possession of salvinorin A and Salvia divinorum. In 2002 we discovered that salvinorin A to be an extrodinarily potent and selective kappa-opioid receptor (KOR) agonist (Roth et al, PNAS, 2002). Salvinorin A's unique chemical structure (it is a diterpene) has facilitated the synthesis of a large number of salvinorin A analogues and the elucidation of salvinorin A's pharmacophore (see Munro et al, J Med Chem 2005 for instance). Some of these analogues have enhanced 'drug-like' properties and may represent candidate medications for a variety of CNS disorders (e.g. chronic pain, depression, psychosis). We have also begun to elucidate the molecular and atomic mechanisms responsible for salvinorin A's selective interactions with KOR's (Yan et al, Biochemistry, 2005 and submitted). Finally, using a modification of the substituted cysteine-accessibility modification (SCAM) technique, we have begun to discover how salvinorin A and related KOR agonists induce receptor activation. Our most recent data suggests an slight twisting of transmembrane segments upon G protein binding. Finally, via collaborative studies we have discovered that the effects of salvinorin A in vivo are abolished by selective ablation of the KOR gene. Taken together, these results demonstrate that salvinorin A is a potent and selective KOR agonist which interacts in a unique manner to KORs. These findings will enhance our ability to exploit KORs for strucure-based drug design of selective KOR agonists and antagonists for the treatment of a variety of psychiatric and non-psychiatric disorders. Supported by grants from NIDA and NIMH.

Kappa Opioid Receptor Activation by Endogenous Opioids Mediates the Dysphoric Properties of Stress

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Background: Salvinorin A has profound effects on mood that presumably result from kappa opioid receptor activation. How kappa receptor activation affects mood is a topic of intense inquiry, and insights to the pharmacological actions of Salvinorin A may be derived from the cellular and molecular analysis of the endogenous kappa/dynorphin system. Prior studies have shown that in C57Bl/6 male mice repeated stress caused by forced swim, neuropathic pain, or social stress results in the release of endogenous dynorphin opioids that activate the kappa opioid receptors. Mice subjected to repeated forced swim stress show a characteristic stress-induced analgesia and stress-induced immobility that are blocked by 1) pretreatment with the kappa antagonist norbinaltorphimine (norBNI), 2) deletion of the kappa opioid receptor (KOR) gene, and 3) deletion of the dynorphin gene. Equivalent effects were also found in male 'intruder' mice subjected to repeated social stress using the Miczek resident-intruder model. Social stress-induced analgesia and social stress-induced defensive behaviors were blocked by norBNI, KOR knockout or DYN knockout. Chronic neuropathic pain caused by sciatic nerve ligation also activated the dynorphin kappa opioid system to induce analgesic effects. Most importantly, swim stress, social stress and neuropathic pain all caused a significant potentiation of conditioned place preference to cocaine that was blocked by disruption of the kappa opioid system.
Methods and Results: To assess the sites of dynorphin action in the brain and spinal cord following stress, we developed an affinity-purified phosphospecific antibody able to distinguish KOR from phospho-serine-369-KOR. We found that agonist activation of kappa opioid receptors initiates an arrestin/G-protein receptor kinase-mediated desensitization process that requires the GRK3 form of the kinase and phosphorylation of the ser-369 in the carboxy tail of the receptor. Behavioral activation of dynorphin release caused an increase in phospho-ser-369-KOR immunoreactivity (KOR-P-ir) detected by western blot analysis and confocal microscopy of mouse brain sections. The increased KOR-P-ir was not evident in chronically stressed mice (swim stressed, social stressed, or nerve-ligated) that had been pretreated with norBNI, or lacking functional KOR or DYN genes. Repeated forced swim stress increased KOR-P-ir in several brain regions and on a variety of cell types characterized by a comprehensive anatomical analysis to be presented in this talk. The goal of this line of research is to define the sites of endogenous dynorphin action within the brain and spinal cord that potentially mediate the analgesic and dysphoric properties. Kappa agonists are known to induce conditioned place aversion, and humans exposed to kappa agonists report feelings of dysphoria. These actions suggest that stress-induced release of endogenous kappa opioids will also produce conditioned place aversion (CPA) and stress-induced dysphoria. To test this hypothesis, mice were exposed to repeated forced swim stress in the presence of a neutral odorant. Following repeated pairing, mice developed CPA to the odorant, and disruption of the kappa opioid systems blocked the CPA. Control experiments demonstrating that kappa antagonism did not block cocaine-odorant induced CPP supports the concept that endogenous dynorphin opioids mediate the dysphoric components of stress.
Conclusion: The studies suggest that kappa agonists are effective analgesic drugs and kappa antagonists may be effective therapeutic tools in treating stress-induced dysphoria and stress-induced drug craving.

Neurobiological Effects of Salvinorin A in Rodents: Implications for the Study and Treatment of Depressive Disorders

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Background: Endogenous opioids appear to play critical a role in the regulation of mood states. For example, there is accumulating evidence that stimulation of kappa-opioid receptors, upon which the endogenous opioid dynorphin acts, can produce depressive-like behaviors in laboratory animals. While depressive conditions can be difficult to model using rodents, numerous tests can be used in parallel to study key signs (including anhedonia, dysphoria, behavioral despair, and anxiety). Here we examined whether systemic administration of salvinorin A (SalvA), a potent and highly selective kappa-opioid agonist, would produce depressive-like effects in the forced swim test (FST), the intracranial self-stimulation (ICSS) test, and the FPS test, which are behavioral models often used to study depressive- and anxiety-like conditions in rats.
Methods: We extracted, isolated, and purified SalvA from *Salvia divinorum* plant leaves, and examined its effects on behavior in the FST, the ICSS test, and the FPS test across a range of doses (0.125-2.0 mg/kg) after systemic (intraperitoneal) administration.

Results: SalvA dose-dependently increased immobility in the FST, an effect opposite to that of standard antidepressant drugs. Doses of SalvA that produced these effects in the FST did not affect locomotor activity in an open field. Furthermore, SalvA dose-dependently elevated ICSS thresholds, an anhedonia-like effect similar to that produced by treatments that cause depressive symptoms in humans (e.g., drug withdrawal, lithium). These effects are virtually identical to those caused by synthetic kappa agonists (U-50488H, U-69593), which are blocked by selective kappa-antagonists. In addition, SalvA also increases FPS, suggesting that kappa-receptors may be involved in the expression of anxiety-like behaviors. At a dose that caused these depressive-like effects in the behavioral assays, SalvA decreased extracellular concentrations of dopamine (DA) within the nucleus accumbens (NAc), a critical component of brain reward circuitry, without affecting extracellular concentrations of serotonin (5HT).
Discussion: These data provide additional support for the hypothesis that stimulation of brain kappa-opioid receptors triggers depressive-like signs in rats, and raise the possibility that decreases in extracellular concentrations of DA within the NAc contribute to these effects. Although SalvA can produce hallucinogenic effects in humans under some conditions (e.g., when smoked), it is conceivable that administration of a derivative or related compound under more carefully controlled conditions might be useful for the treatment of disorders characterized by hyperfunction of dopamine systems (e.g., mania). When considered with previous evidence indicating that kappa-antagonists have antidepressant-like effects in rodents, this line of research suggests that kappa-receptor ligands could represent a new approach in the management of mood disorders. Furthermore, it raises the possibility that dysregulation of brain dynorphin and/or kappa-receptor function may contribute to the etiology and pathophysiology of prominent psychiatric conditions.

Salvinorin A: Behavioral and Neuroendocrine Effects

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Background: The plant-derived hallucinogen salvinorin A was recently shown to be a selective kappa-opioid agonist, demonstrating high efficacy in vitro (Roth et al., 2002). Systematic data on the in vivo pharmacology of salvinorin A in humans are limited, therefore data on the effects of salvinorin A in experimental animals are a useful background to understand the pharmacology of this compound. Several relatively well characterized assays have been previously used to quantitatively study the in vivo effects of kappa-opioids in non-human primates.
Methods: Salvinorin A was studied in non-human primates (adult male or female rhesus monkeys; N=3-4 per study), by the subcutaneous or intravenous routes. Salvinorin A was studied in behavioral and neuroendocrine assays previously shown to be sensitive to structurally diverse kappa-opioid agonists.
Results: Salvinorin A (0.0032-0.032 mg/kg) was generalized by non-human primates trained to discriminate the centrally-penetrating synthetic kappa-agonist U69,593; this effect was blocked by the opioid antagonist quadazocine (0.32 mg/kg). Salvinorin A (i.v.) produced dose-dependent unresponsiveness to environmental stimuli (sedation-like), as measured with "blind" observational rating. This effect exhibited a fast onset (by 5 min) but short duration (e.g., declined before 30 min), similar to the presumed duration of salvinorin A-containing preparations in humans. This effect could be prevented and apparently reversed by opioid antagonism (with naltrexone or nalmefene). Similar to other kappa-agonists, salvinorin A (0.0032-0.032 mg/kg; i.v.) caused robust dose-dependent increases in serum prolactin levels in monkeys. For example, with a dose of 0.032 mg/kg salvinorin A (i.v.), prolactin rose from mean baseline pre-injection levels of approximately 11 ng/ml to mean levels of approximately 170 ng/ml, 15 min post-injection. This indicates a salvinorin A-induced modulation of dopaminergic hypothalamic tone, since the latter has inhibitory control over prolactin release. This neuroendocrine effect of salvinorin A was sensitive to blockade by the opioid antagonist nalmefene (0.1 mg/kg).
Conclusion: Overall, salvinorin A exhibits behavioral and neuroendocrine effects qualitatively similar to those of synthetic kappa-agonists in non-human primates. This supports the hypothesis that the powerful in vivo effects of salvinorin A in humans (e.g., perceptual and emotional disturbances) are mediated by the kappa-opioid receptor system. This work was funded by NIH-NIDA grants DA05130 and DA11113 and DA017369.

Panel Session
5HT2A Receptor as a Potential Therapeutic Target for Depression and Suicide: Evidence from the Bench

Cortical 5-HT2A Receptor Signaling Modulates Risk Assessment and Behavioral Disinhibition in Mice: Potential Links to Suicidal Behavior

Jay Gingrich*, Noelia V. Weisstaub, Mingming Zhou, Alena Lira, Evelyn Lambe, Javier González-Maeso, Jean-Pierre Hornung, Etienne Sibille, Mark Underwood, Shigeyoshi Itohara, William T. Dauer, Mark S. Ansorge, Emanuela Morelli, J. J. Mann, Miklos Toth, Stuart C. Sealfon and George Aghajanian

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Serotonin neurotransmission in the central nervous system modulates depression and anxiety-related behaviors in humans and rodents but the responsible downstream receptors remain poorly understood. Here we demonstrate that global disruption of 5-HT2A receptor (5HT2AR) signaling in mice reduces inhibition in conflict anxiety paradigms without affecting fear-conditioned and depression-related behaviors. Selective restoration of 5HT2AR signaling to the cortex normalized conflict anxiety behaviors. These findings indicate a specific role for cortical 5HT2AR function in the modulation of conflict anxiety—consistent with models of cortical, "top-down" influences on risk assessment. A role for cortical 5HT2AR signaling in risk assessment and behavioral disinhibition supports a possible role of 5HT2AR dysregulation in suicidal behavior.

5HT2A Receptors in Depression and Suicide: Possible Targets for Development of Antidepressants Drugs

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Serotonergic abnormalities are implicated in depression and suicide, primarily based on observations of lower 5-hydroxyindoleacetic acid in CSF of suicidal patients. Neuroendocrine studies have indicated abnormalities in 5HT1A and 5HT2A in depression and suicide. SSRIs supposedly produce their antidepressant effects by inhibiting the uptake of serotonin leading to changes in serotonin receptor linked signaling systems in the brain. However, SSRIs are not effective in the treatment of all types of depression and suicidal behavior. If we know the specific site of serotonergic abnormalities associated with depression and/or suicide, this knowledge may be used to develop specific and more effective therapeutic interventions. Abnormalities of 5HT2A receptors have been observed in the postmortem brain of suicides. However, some investigators find no differences in 5HT2A receptors between normal controls (NC) and suicidal subjects. This is primarily because radioligands used for labeling 5HT2A receptors are nonspecific and also label other receptors. To clarify this inconsistency and if the 5HT2A receptors are altered in the postmortem brain of suicides, we determined not only the binding sites for 5HT2A receptors using [125I]LSD as a ligand, but we also determined the protein and mRNA expression of 5HT2A receptors in prefrontal cortex (PFC), hippocampus and nucleus accumbens obtained from teenage suicides and matched NCs. We observed that [125I]LSD binding was increased in PFC of teenage suicides as compared to NCs. The protein

expression of 5HT2A receptors as determined by Western blot technique was significantly increased in PFC (Brodman Area 9) of suicides as compared to NC but not in nucleus accumbens. This increase in protein expression was associated with a concomitant increase in the 5HT2A receptor mRNA levels in PFC and hippocampus of teenage suicides as compared to NCs. To examine the specific localization of this increase in protein expression of 5HT2A receptors, we used immunohistochemistry and observed that this increase in 5HT2A receptors occurred in the pyramidal cells of Layer V in PFC of teenage suicides as compared to NCs. 5HT2A receptors are also richly distributed in the platelets and we therefore determined 5HT2A receptors in the platelets of depressed, manic and schizoaffective patients with or without suicidal behavior using [125I]LSD as ligand. 5HT2A receptors were increased in the platelets of depressed, bipolar and schizoaffective patients as compared to NCs; however, this increase was most pronounced in suicidal patients. Taken together, these studies indicate significant increase in the expression of 5HT2A receptors in brain and platelets of depressed and suicidal subjects. It is therefore quite possible that targeting 5HT2A receptors for developing therapeutic interventions may be useful in the treatment of depression as well as suicidal behavior.

Serotonin 2A (5-HT2A) Receptor Elevations and Serotonin Lowering Mechanisms During Major Depressive Episodes

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Using [18F] setoperone positron emission tomography (PET), we found that during major depressive episodes, subjects with greater severity of dysfunctional attitudes (more severe pessimism) had a 29 per cent greater prefrontal cortex 5-HT2A receptor binding potential (Meyer et al. Am J Psych 2003). The binding potential (BP) is an index of receptor density. This in vivo finding shows consistency with postmortem reports of greater prefrontal 5-HT2A density in suicide victims: A high proportion of suicide victims are in the midst of a major depressive episode. Higher risk for suicide is associated with greater hopelessness and hopelessness is consistently correlated with severity of dysfunctional attitudes. Thus investigations reporting greater prefrontal 5-HT2A density in suicide victims may be sampling major depressive episodes with more severe pessimism (or dysfunctional attitudes). Cortex 5-HT2A receptor density increases after chronic serotonin depletion and decreases following chronically raised serotonin. It is proposed that elevated prefrontal 5-HT2A BP during major depressive episodes is secondary to loss of extracellular serotonin. One proposed mechanism for serotonin loss is via elevated 5-HTT density: Using [11C] DASB PET, we found that prefrontal 5-HTT BP was elevated 27 per cent during depressive episodes with severe levels of dysfunctional attitudes (pessimism) (Meyer et al. Arch Gen Psych 2004). A second proposed mechanism for serotonin loss is via greater MAO-A function. Using [11C] harmine PET, we found that MAO-A DVs, an index of MAO-A density was elevated more than two standard deviations (30 per cent) in prefrontal cortex during major depressive episodes (Meyer et al. Arch Gen Psych in press). These studies collectively sample 71 subjects in the midst of major depressive episodes, who were medication free for at least 4 months, and had early onset major depressive disorder. In this specific illness type and state, elevated prefrontal 5-HT2A BP is likely consequent to lower extracellular serotonin. Therefore, during major depressive episodes of early onset major depressive disorder, raising extracellular serotonin is logically appropriate to target elevated prefrontal 5-HT2A density.

Dysfunctional 5-HT2A Receptor Binding in Depression: A Therapeutic Target for Drug Action in Recurrent Depression?

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Background: Recent work from the STAR*D trial has suggested that the 5-HT2A receptor may be a therapeutic target for the treatment of depression. The purpose of the present study was to use the highly selective 5 HT2A receptor ligand, [11C]MDL 100,907, in a PET imaging paradigm, to assess 5 HT2A receptor binding potential in euthymic subjects with a history of recurrent depression and to assess the relationship between receptor binding and scores on the dysfunctional attitudes scale (DAS).
Methods: Cortical 5-HT2A receptor binding was measured in 20 unmedicated, fully recovered unipolar depressed patients and 20 age and gender-matched controls using [11C]MDL 100,907 PET. Regional estimates of binding potential (BP) were obtained using a reversible plasma input function compartmental model and the cerebellum as a reference region to estimate the free and non-specifically bound [11C]MDL 100,907 in brain tissue.
Results: Relative to the controls, the recovered depressed patients demonstrated significantly higher 5 HT2A receptor binding potential in frontal cortex (mean increase 19%), parietal cortex (mean increase 25%) and occipital cortex (mean increase 19%). 5-HT2A receptor binding potential correlated negatively with age in both patients and controls and positively with the DAS in the recovered patients.
Conclusions: Recovered subjects with a history of recurrent major depression have elevated binding potential of cortical 5-HT2A receptors. The correlation of increased 5 HT2A receptor with increased scores on DAS supports earlier work suggesting that increased 5 HT2A receptor availability characterises a group of depressed patients with high levels of dysfunctional attitudes. The 5-HT2A receptor may therefore be a potential therapeutic target for the development of new molecules for the treatment of recurrent depression.

Panel Session
Can Correcting Cognitive Deficits Improve Treatment Responses in Substance Abusing Patients?

Endogenously Active Molecular Targets for Drug-Induced Cognitive Enhancement

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Background: Although there have been many molecular and synaptic mechanisms postulated to have relevance for learning and memory, few have actually been observed to show endogenous activation during associative memory paradigms such as Pavlovian conditioning or spatial maze learning. Non-associative phenomena such as habituation, sensitization and LTP, for example, have, for the most part, not generated drugs that specifically enhance cognition (i.e., associatve memory) even in animal models, let alone human trials. To offer drug abuse patients the benefit of enhanced learning ability during, for example, an extinction therapy protocol, it may be more productive to identify molecular and synaptic events that are endogenously activated during associative learning. A critical pathway that we have identified involves the PKC isozymes activated by the associatively activated second messengers calcium, diacylglycerol, and arachidonic acid.
Methods: Biochemical and molecular biologic assays of sequential substrate involvement have included autoradiographic labels of membrane-associated PKC isozymes, in situ measures of mRNA levels in brain structures and neuronal ensembles, immunohistochemical assays of protein levels in individual neurons within neuronal populations, microarray screening for individual gene activation and RT-PCR. Electrophysiologic measures of memory-specific changes of synaptic function have included intra- and extracellular recording, voltage-clamp and patch-clamp methodologies.
Results: PKC isozymes alpha and epsilon were found during associative learning to activate a sequence of downstream targets that include the Type II ryanodine receptor, MAP kinase Erk1/2, the ELAV mRNA stabilizing proteins HuD and HuC, carbonic anhydrase and other proteins engaged in synaptic plasticity and/or remodelling such as GAP43. These PKC isozyme pathways were, in turn, regulated by

factors such as the cbl-b protein, FGF 18, BDNF, and the insulin signaling receptors. Synaptic facilitation of EPSPs and disinhibition from GABAergic IPSPs have been demonstrated to be functionally controlled during memory consolidation by these PKC isozyme pathways. Drugs have now been identified, such as the macrolactone, Bryostatin, and the imidazole carbonic anhydrase activators that show remarkable specificity for enhancing sequential steps in the associative memory process including attention, acquisition, consolidation, and long-term memory.
Discussion: These drugs are now being pursued as cognitive enhancers to ameliorate addictive conditioning as well as other neurologic disorders, such as Alzheimer's disease. The cognitive enhancing potency of such drugs as Bryostatin as well as their lack of toxicity will critically depend on the molecular precision of their effects within the identified signaling pathways that are endogenously activated during associative learning and memory paradigms.

D-Cycloserine, a NMDA Agonist, Facilitates Extinction of a Cocaine-Induced Conditioned Place Preference in Rats
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A particularly salient feature of substance abuse is the persistence of the ability of drug-associated environmental cues to elicit drug craving and consequently reinstate drug seeking and drug taking. The capacity of drug-related cues to reinstate drug craving has been shown to be resistant to extinction. Their efficacy is persistent and can last several weeks, even months, in the absence of re-exposure. In view of the long-lasting effects of drug-paired cues, the facilitation of extinction of responses elicited by such cues could help drug addicts avoid or at least reduce their experience of craving after the period of detoxification. There are several reports that systemic or intra-amygdala administration of D-cycloserine, a partial agonist at the glycine site on the NMDA receptor, facilitates extinction of learned fear induced by a conditioned stimulus. These effects of D-cycloserine have been reported only for aversive conditioning. In the work to be described, we investigated the effect of administration of D-cycloserine on extinction of an appetitive conditioning, a cocaine-induced conditioned place preference. Male Long Evans rats were given four 20-min pairings in one outer chamber of a 3-compartment box with cocaine (20 mg/kg, i.p.) and four pairings of the other outer chamber with saline on alternate days. During extinction sessions, drug-free rats were placed in the center choice chamber with access to the entire apparatus for 15 min test once a day for 10 consecutive days and the time spent in each compartment was recorded. At the end of the conditioning, all rats exhibited a significant preference for the compartment previously associated with cocaine. Immediately following this and each subsequent extinction test rats were injected i.p. with saline or with D-cycloserine (15 mg/kg). Saline-treated rats maintained the preference for the compartment previously paired with cocaine for at least five days of extinction. When rats were treated by an injection of D-cycloserine immediately after each extinction trial, they required fewer sessions to extinguish their preference. Similar data were obtained when D-cycloserine was injected directly into basolateral nucleus of the amygdala bilaterally (10 microg/0.5 microl/side), immediately after sessions 1, 3 and 5 of extinction. Such findings were not observed when D-cycloserine was injected peripherally 4 hours after the end of extinction sessions. To our knowledge, these experiments are the first to demonstrate that D-cycloserine facilitates extinction of a conditioned appetitive response, a cocaine-induced conditioned place preference, apparently by enhancing acquisition and/or consolidation of new learned associations during extinction training by actions at NMDA receptors in the amygdala. These data suggest that D-cycloserine could be used to help human drug addicts extinguish the emotional and physiological responses and thoughts induced by environments and cues previously associated with drug use. As a result, these findings may contribute to the development of pharmacological therapies to avoid or at least reduce craving in humans during the periods of detoxification and abstinence.

Chronic Substance Abusers: Are There Possibilities for Cognitive Enhancement?
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Recent work by ourselves and many other groups show that chronic substance use can induce impairment across a broad range of cognitive processes including learning and memory, and fronto-executive functions of planning, cognitive flexibility, decision making and response inhibition. As indicated by parallel studies utilising structural and functional neuroimaging, these mnemonic and executive functions are subserved by neural networks that include relatively greater involvement of posterior and anterior cortical regions respectively, such as the hippocampal formation and prefrontal cortex. Many of the cognitive deficits are residual in former drug users, suggesting that the neurocognitive impairment does not simply reflect the current effects of substances abused, but may reflect neurotoxic actions of the drugs employed or a premorbid state or trait associated with cognitive dysfunction. Impairments in impulse control, decision-making and cognitive flexibility frequently translate into behavioural problems of erratic and ill-judged behaviour as exemplified by the sharing of needles, the increased frequency of accidents and tendencies to indulge in other risky behaviours, such as driving under the influence of drugs. In this talk we consider the possibility that at least some of these deficits may be remediated using cognitive enhancers, such as modafinil. We will review our recent work showing cognitive enhancement produced by modafinil in some cognitive domains including the control of impulsivity (stop-signal reaction time task), planning (Tower of London problem solving), cognitive flexibility (ID/ED Attentional Shift) and memory, not only in normal control volunteers, but also in adult patients with attention deficit/hyperactivity disorder and schizophrenia. The molecular mode and neural locus of action of modafinil remains unclear, although the drug is licensed for the treatment of narcolepsy. It has many of the major effects of classical stimulant drugs of abuse, although it does not appear to have abuse potential. We will also consider other options for cognitive enhancement. Acknowledgements - This work was funded in part by the Wellcome Trust and the Medical Research Council (UK).
Declaration – Consultant for Cambridge Cognition Ltd.

Cognitive Deficits in Stimulant Abusers: Types of Deficits and Their Possible Modulation
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Substance abuse treatment is characterized by high dropout rates and less than optimal treatment responses. Recent evidence suggests that poor cognitive functioning may have a major impact on treatment retention in cocaine users (Aharonovich et al, Drug and Alcohol Dependence 2006). Similarly, high levels of impulsivity on the Barratt Impulsivity Scale are negatively correlated to treatment retention in cocaine users (Moeller J Subst Abuse Treatment 2001). A recent observational study of methamphetamine users in treatment in California noted that only 60 % completed treatment (Hser et al J Subst Abuse Treatment 2005). A moderately high proportion of patients in this study complained of depression (35 %), severe anxiety (40 %), having trouble comprehending or remembering material (31 %), and trouble controlling violent behavior (12 %). The findings are buttressed by studies of amphetamine and methamphetamine users who have undergone neuropsychological testing. Deficits in attentional processes, working memory, recall, strategic thinking, impulsivity and decision-making have been reported. Perseveration errors and inability to ignore irrelevant stimuli have been reported. The inability to ignore irrelevant

stimuli is posited to be responsible for the distractability seen in stimulant dependent patients. Some of the attentional processing deficits and perseveration error deficits can be modeled in animals given amphetamine, suggesting that chronic amphetamine administration may produce such deficits. The cognitive deficits will be reviewed and possible pharmacological modulation of these deficits will be presented in this presentation and the rest of the presentations in the Panel.

Panel Session
Epigenetics of Psychiatric Disorders

Epigenetics of Memory Storage

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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 expression 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. We have examined mice in which CBP activity in neurons is reduced either by the transgenic expression of an inhibitory form of cbp lacking the HAT domain or by knocking in a mutation of the CREB transcription factor-binding KIX domain of cbp. This genetic approach enabled us to compare the role of CBP-associated HAT activity in memory and synaptic plasticity with the role of the KIX transcription factor-binding domain of CBP. We found that mutant mice expressing an inhibitory form of cbp exhibit impairments in spatial and contextual memory and in long-lasting forms of hippocampal synaptic plasticity. KIX knock-in mice were also observed to have significant impairments in contextual memory. A complementary method to study the role of histone acetylation in synaptic plasticity and memory is to examine the effects of HDAC inhibitors, which increase the level of histone acetylation that correlates with transcriptional activation. We found that increasing histone acetylation using the HDAC inhibitor TSA enhances long-term contextual memory and facilitates synaptic plasticity via the transcription factor CREB. In summary, these results support the idea that histone acetyltransferases and histone acetylation are critical to mechanisms of long-term memory storage. Histone acetylation may provide an epigenetic mechanism for establishing gene-specific modifications that result in the coordinate expression of genes required for long-term memory storage.

Role of Chromatin Remodeling in Drug Addiction

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Given that cocaine induces adaptations in brain by regulating gene expression, we are interested in the role played by chromatin remodeling at specific gene promoters as a key underlying mechanism involved. Recently, we used chromatin immunoprecipitation (ChIP) assays to show that cocaine induces specific histone modifications at different gene promoters in rat striatum, a major neural substrate for cocaine's behavioral effects. At the cFos promoter, H4 hyperacetylation is seen within 30 min of a single cocaine injection, whereas no histone modifications were seen with chronic cocaine, consistent with cocaine's ability to induce cFos acutely but not chronically. In fact, histones at the cFos promoter undergo methylation after chronic cocaine and this is associated with desensitization of the gene. In contrast, at the BDNF and Cdk5 promoters, two genes that are induced by chronic but not acute cocaine, H3 hyperacetylation was observed with chronic cocaine only. DeltaFosB, a cocaine-induced transcription factor important for in the addiction process, appears to mediate this regulation of the Cdk5 gene. In contrast, a distinct transcription factor, CREB, appears to mediate regulation of the BDNF gene. Furthermore, we have found that inhibiting histone deacetylase (HDAC) activity enhances the locomotor and rewarding responses to cocaine, whereas overexpressing certain HDACs specifically within striatum, by use of viral-mediated gene transfer, has the opposite effect. We hypothesize that chromatin remodeling is an important regulatory mechanism underlying cocaine-induced neural and behavioral plasticity. We are now extending these studies in three main ways. First, we are using ChIP on chip assays, where chromatin immunoprecipitates are analyzed on promoter DNA microarrays, to obtain a more global analysis of genes in striatum which show activation (histone acetylation) or repression (histone methylation). Second, we are determining which of these changes in chromatin structure at cocaine-regulated genes are mediated via DeltaFosB, CREB, or other drug-regulated transcription factors. These experiments are enabled by the availability of mutant mice, in which DeltaFosB or CREB—or dominant negative antagonists of these transcription factors—can be induced in the striatum of adult animals. Thus, by use of these mice, followed by ChIP on chip assays using CREB or DeltaFosB immunoprecipitations, it will be possible to identify the "transcriptomes" of these transcription factors in striatum and their contribution to the genomic effects of cocaine. Third, we are exploring the functional significance of these changes with further behavioral studies. Our initial work utilized non-specific HDAC inhibitors administered systemically. We are now administering the inhibitors directly into the striatum to show that the enhancement in cocaine's behavioral effects is mediated by this brain region, as we hypothesize, and use more specific inhibitors as they become available. In addition, we are using viral vectors to overexpress a range of HDACs to determine if there is specificity in terms of their ability to oppose behavioral responses to cocaine. Together, this work is establishing the importance of chromatin remodeling in the long-term actions of cocaine.

Dopaminergic Signaling Induces Chromatin-Remodeling in Striatal Neurons

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Stimulants, antipsychotics and antidepressants interacting with monoamine signaling pathways induce changes in gene expression and metabolic activity in multiple brain regions. The underlying molecular mechanisms are likely to include acetylation and other covalent modifications of nucleosome core histones, which regulate chromatin function and transcription. Here, we show striatal neurons dynamically regulate histone acetylation and phospho-acetylation by dopaminergic and glutamatergic inputs converging on the cellular level. Furthermore, pre-treatment of rats with sodium butyrate, a short acting inhibitor of histone deacetylase enzymes, resulted in a striking increase in striatal BOLD-MRI (blood-oxygen-level-dependent magnetic resonance imaging) signals after repeated exposure to cocaine. These finding suggest that chromatin modifying drugs with opposing effects on histone acetylation, including histone deacetylase and acetyltransferase inhibitors (HDACi and HATi) may differentially affect stimulant-induced changes in gene expression, brain activation and addiction behavior.

Epigenetic Mechanisms in the Transmission of Behaviour Across Generations

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In both primates and rodents there is evidence for the transmission of maternal behaviour from one generation to the next such that moth-

ers who display high levels of contact with offspring or in the case of rodents, display high levels of maternal licking/grooming (LG), produce female offspring who display this trait toward their own offspring. Cross-fostering studies suggest that this transmission is mediated by the postpartum environment. Investigation of the neural mechanisms involved in the expression of individual variation in maternal care in rodents suggests that levels of estrogen receptor mRNA in the medial preoptic area (MPOA) of the hypothalamus are associated with frequency of postpartum LG in lactating dams. This differential mRNA expression is also observed in the female offspring of these mothers both at 6-days of age and in adulthood. We have explored the role of epigenetic modifications in mediating these long-term changes in gene expression. DNA methylation is an epigenetic mechanism of gene silencing which prevents the binding of transcription factors to response elements. Analysis of the promoter region of the estrogen receptor in these females indicates that offspring of high LG dams have decreased cytosine methylation in the MPOA compared to offspring of low LG dams. This differential methylation occurs at several regions within the promoter, including a Stat5 response element. Chromatin immunoprecipitation assay confirms reduced binding of Stat5 to the estrogen receptor alpha promoter region in female offspring of low compared to high LG dams. These results indicate that the transmission of maternal LG from mother to offspring is associated with epigenetic changes which alter gene expression in infancy and are maintained into adulthood. This mechanism may result in the behavioral transmission of many aspects of phenotype, particularly those traits that provide an adaptive advantage to offspring.

Panel Session
Kynurenic Acid: A New Player in the Pathophysiology of Schizophrenia

Bidirectional Regulation of Extracellular Dopamine by Endogenous Kynurenate in Striatum and Prefrontal Cortex

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Kynurenic acid (KYNA) is a major metabolite of tryptophan degradation. In the brain, KYNA is synthesized in astrocytes and then released into the extracellular compartment. At physiological (nanomolar) concentrations, KYNA inhibits the glycine co-agonist ("glycineB") site of the NMDA receptor and, in particular, is a very potent antagonist of the α7 nicotinic acetylcholine (α7nACh) receptor. These properties raise the possibility that astrocyte-derived KYNA serves as an endogenous neuromodulatory agent. Our laboratory uses in vivo microdialysis in unanesthetized rodents to examine such a possible role of KYNA. Because of the fact that KYNA levels are increased in the basal ganglia and cortex of patients with schizophrenia, we have focused our attention primarily on the effects of KYNA in these brain regions and, in particular, on the possible regulation of extracellular dopamine (DA) levels. Focal intracerebral infusion of nanomolar concentrations of KYNA reduces basal extracellular DA levels in the striatum and prefrontal cortex of rats. In the striatum, this effect is initiated by the KYNA-induced inhibition of α7nACh receptors on glutamatergic afferents. Conversely, extracellular DA levels in both brain areas are enhanced when KYNA formation is compromised by local perfusion with specific inhibitors of kynurenine aminotransferase II (KAT II), a major biosynthetic enzyme of brain KYNA. This is also in line with studies in the striatum of mice with a targeted deletion of KAT II, where extracellular KYNA levels are substantially reduced and extracellular DA levels are significantly increased. Taken together, a picture emerges where fluctuations in the astrocytic production of KYNA inversely regulate dopaminergic tone under physiological conditions. Studies in the rat striatum indicate that this regulation of extracellular DA levels by KYNA extends to the effects of the psychostimulant d-amphetamine (d-Amph). Thus, unilateral intrastriatal infusion of nanomolar concentrations of KYNA completely prevents the substantial increase in extracellular DA observed in the vehicle-infused contralateral striatum following the systemic administration of d-Amph (5 mg/kg). This KYNA-related imbalance in DA between the two striata causes animals to rotate towards the side of the KYNA infusion for the duration of the d-Amph effect. Interestingly, the ability of KYNA to abolish the d-Amph-induced DA release is duplicated by the specific α7nACh receptor antagonist methyllicaconitine (MLA) but not by the specific glycineB receptor antagonist 7-Cl-KYNA. This indicates that α7nACh inhibition, but not glycineB receptor blockade, mediates the reduction of d-Amph-induced DA release by KYNA in the striatum. In summary, the link between KYNA and DA is not only of obvious relevance for the pathophysiology of schizophrenia but has broader implications for drug addiction and other clinical conditions associated with, and possibly etiologically linked to, a defective reward system. Since fluctuations in tonic, extracellular DA levels influence neuronal vulnerability to excitotoxic injury, the relationship between KYNA and DA also ought to be scrutinized in relation to excitotoxic diseases and conditions such as Huntington's disease, cerebral ischemia and severe hypoglycemia.

Role of Endogenous Kynurenic Acid in the Control of Dopaminergic Neurotransmission

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In recent years progress in the field of schizophrenia research has led to the suggestion that dopamine only plays an intermediary role in the pathophysiology of the disease and that the main abnormalities lie elsewhere. In this regard deficits in brain glutamatergic systems have gained growing interest. Kynurenic acid (KYNA) is an endogenous antagonist at the alpha7-nicotinic receptor as well as a glutamate receptor blocker with a preferential action at the glycine-site of the N-methyl-D-aspartate (NMDA)-receptor. Mounting evidence indicate that the compound is significantly involved in basal neurophysiological processes in the brain. Thus, pharmacologically elevated levels of KYNA, in similarity to systemic administration of NMDA receptor antagonists (e.g. PCP, MK 801), are associated with increased neuronal activity of midbrain dopamine neurons. In addition, lowering of brain KYNA is associated with a reduced firing, suggesting that midbrain dopamine neurons are tonically driven by endogenous KYNA. Moreover, in a large cohort of male patients with schizophrenia (n = 90), we recently confirmed that levels of KYNA are elevated in the cerebrospinal fluid (CSF) from first-episode, drug-naïve patients as well as from patients on antipsychotic treatment. Furthermore, in male patients with schizophrenia as well as in healthy volunteers, CSF KYNA was positively correlated to the CSF concentration of homovanilic acid (HVA), the main metabolite of dopamine. In summary, our results suggest that KYNA tonically modulates glutamatergic mechanisms thereby controlling brain dopaminergic neuronal activity. The elevated CSF KYNA observed in patients with schizophrenia may reflect a state of increased KYNA turnover in the brain.

Developing a Model of Kynurenic Acid Effects on Information Processing

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Background: Prepulse inhibition (PPI) of the acoustic startle reflex refers to the ability of a weak stimulus preceding a startling stimulus

to inhibit the response to that stimulus. PPI is an operational measure of sensorimotor gating that is amenable to cross-species comparisons. Deficits in PPI have been reported repeatedly in patients with schizophrenia and other psychiatric disorders characterized by abnormalities in sensory, cognitive, or motor gating. Similar deficits in PPI can be produced in rodent models through pharmacological or developmental perturbations. Isolation rearing of rats from weaning is a non-pharmacological manipulation that leads to deficits in PPI and has been used as a neurodevelopmental model to study the pathophysiology of schizophrenia. Additionally, administration of NMDA receptor antagonists, such as PCP, leads to deficits in PPI that mimic those observed in schizophrenia patients. Kynurenic acid (KYNA) is an endogenous glutamate-receptor antagonist, preferentially blocking NMDA receptors. Recent studies have shown that endogenous levels of KYNA are elevated in the cerebrospinal fluid or post-mortem brains of schizophrenia patients. Hence, the present studies examined the effects of KYNA on startle and PPI in pharmacological and developmental rodent models.

Methods: We first conducted acute pharmacological studies assessing the effects of elevations in KYNA on startle and PPI in rats. Specifically, we administered the precursor of KYNA (l-kynurenine) or PNU 156561A, which blocks kynurenine 3-hydroxylase (shifting synthesis of kynurenine toward KYNA), to rats and assessed PPI. We also tested whether PPI deficits produced by pharmacologically induced elevations of KYNA were reversed by the antipsychotic drugs haloperidol and clozapine. Based on the observation of elevated KYNA in the brains of schizophrenia patients, we then examined levels of KYNA in the brains of isolated and socially housed rats. In this experiment, rats were assigned to social or isolation housing at weaning and then tested in the acoustic startle/PPI paradigm 8 weeks post-weaning. After behavioral testing, brains were removed and dissected for determination of KYNA.

Results: Pharmacologically induced elevations of KYNA produced by administration of l-kynurenine or PNU 156561A disrupted PPI in rats. These disruptions in PPI were prevented by both the typical antipsychotic haloperidol and the atypical antipsychotic clozapine. In the second set of experiments, isolated rats exhibited deficits in PPI compared to socially housed controls. Similar to the finding in schizophrenia patients, KYNA levels were elevated in the hippocampus of isolated rats.

Conclusion: The pharmacological data showed that elevations of KYNA disrupt PPI in rats similar to that observed with other NMDA antagonists such as PCP and that these PPI disruptions can be ameliorated by antipsychotic drugs. The developmental data identify a neurochemical link between isolation rearing and specific neuropathological findings in schizophrenia. Taken together, these findings suggest that KYNA serves as an endogenous modulator of PPI and that elevated levels of KYNA may be a potential mechanism through which isolation rearing exerts its effects on sensorimotor gating in rats. These preclinical studies using rodent models of PPI provide further evidence for the potential role of KYNA in schizophrenia. Future studies will examine whether the effects of KYNA on sensorimotor gating in developmental models is due to acute or chronic elevations of KYNA. Supported by MH52885.

Interactions Between Kynurenic Acid and the Nicotinic Allosteric Potentiating Ligand Galantamine: Relevance for Treatment of Schizophrenia

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Background: Kynurenic acid (KYNA), a neuroactive metabolite of the kynurenine pathway in the brain, is a non-competitive antagonist at α7 nicotinic receptors (nAChRs; J Neurosci 21:7563, 2001). We have provided evidence that the N-terminal domain of the α7 nAChR subunit contains the recognition sites for KYNA (J Neurobiol 53:479, 2002). The binding region for the nicotinic allosteric potentiating ligand galantamine, an FDA-approved drug for treatment of Alzheimer's disease currently being tested in clinical trials as an adjuvant treatment for schizophrenia, is also located on the N-terminal domain of nAChR α subunits (J Neurobiol 53:479, 2002). Therefore, this study was designed to evaluate possible interactions between KYNA and galantamine on α7 nAChRs.

Methods: Whole-cell currents were evoked by application of the α7-nAChR agonist choline (1 mM) to cultured hippocampal neurons under control conditions and in the presence of KYNA and/or galantamine. The effect of KYNA alone or in the presence of galantamine on choline-triggered GABA release was also examined in whole-cell, voltage-clamped striatal or hippocampal neurons in culture.

Results: In cultured hippocampal neurons, KYNA (0.01-100 μM) caused a concentration-dependent, voltage-independent reduction of the amplitudes of choline-evoked whole-cell currents. The effect could not be easily reversed by simply washing the neurons with KYNA-free physiological solution. In contrast, galantamine had a biphasic effect on α7 nAChRs in hippocampal neurons. At concentrations ranging from 0.3 to 3 μM galantamine increased voltage independently the amplitude of choline-evoked whole-cell currents. At higher concentrations, galantamine caused a voltage-dependent inhibition of α7 nAChRs. The effects of galantamine on α7 nAChRs were promptly reversed upon washing the neurons. When the neurons were first exposed to galantamine (1 μM) and then to galantamine-plus-KYNA (0.01-100 μM), KYNA caused less inhibition of choline-evoked currents than it did in the absence of galantamine. The finding that galantamine caused a rightward shift in the concentration-response relationship for KYNA-induced inhibition of α7 nAChR activity strongly suggested that the binding regions for galantamine and KYNA on α7 nAChRs overlap. Application of choline (1 mM) to hippocampal or striatal neurons triggered inhibitory postsynaptic currents (IPSCs) in the continuous presence of glutamate receptor antagonists (CNQX, 10 μM and APV, 100 μM). In both hippocampal and striatal cultures, choline-triggered GABA release was blocked by the α7 nAChR antagonist α-bungarotoxin (100 nM), and thereby resulted from the activation of α7 nAChRs on GABAergic neurons synapsing onto the neurons from which recordings were obtained. Choline-triggered IPSCs were reversibly blocked by KYNA (10 μM), and the magnitude of the effect of KYNA was significantly reduced by pre- or post-exposure of the neurons to galantamine (1 μM).

Discussion: The ability of galantamine to counteract the inhibitory actions of KYNA on nAChRs lends support to the concept that galantamine will be a useful ajuvant therapy for treatment of schizophrenia, a neuropsychiatric disorder in which brain KYNA levels are known to be elevated and α7 nAChR activity is impaired.

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Panel Session
New Frontiers in Imaging Phasic Dopamine Release in Humans

Imaging Intrasynaptic Dopamine Release: Concepts, Challanges and Contributions

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While neuroreceptor mapping with PET/SPECT are now a mature methodology, an often touted contribution of this in vivo imaging over ex vivo/in vitro procedures has been the ability to carry out serial studies for disease or treatment progress and to measure receptor occupancy. For the latter measures it is not only to examine therapeutic drug PK/PD and aid rational drug dosing but also to measure endogenous neurotransmitters (NT) occupancy by competition with

the receptor binding PET/SPECT radioligand. This measurement of NT concentration and release is the most novel aspect of these in vivo studies compared to other neuroscience techniques. Even microdialysis cannot directly measure intrasynaptic NT concentrations and competition with radiotracers. We will briefly review the animal and human history of NT competition with radioligand binding in the dopamine (DA)system with methylphenidate and amphetamine stimuli as well as the DA-depleters reserpine and AMPT. This talk will review the current state of these methods in studying various neuropsychiatric disorders such as Tourette's (TS), Restless Leg Syndrome (RLS), Alcoholism, Cocaine use, Schizophrenia, and ADHD. The possible role of new radiolabelled dopamine agonists as opposed to antagonists and possible future application for imaging different dopamine affinity states will be discussed. Finally, the talk will develop the pros and cons of using psychostimulants as challenge studies in human research. Lastly, this talk will introduce other multiple modulator systems such as the GABA and glutamate systems to be discussed by the other speakers. Examples of data to be discussed: Using [^{11}C]Raclopride PET, we have studied both cue- and stimulant-induced dopamine release (DA_{rel}) in TS, RLS, schizophrenia, alcohol and drug abuse. The largest difference reported has been in TS, where TS patients have 100% greater amphetamine (AMP) induced DA_{rel} than controls: 8% in 10 controls vs. >16% DA_{rel} in 16 age-matched TS. In a study of cue-induced cocaine craving, there was a DA_{rel} increase of 5-10% when chronic cocaine abusers reported craving during the presentation of audiovisual drug cues (N=11) over cocaine users that did not report craving (N=8). With AMP induced DA_{rel}, chronic cocaine users had 100% lower DA_{rel} than controls: 9-10% DA_{rel} in 12 controls vs. 0-2% in 17 age-matched cocaine users. Alcoholics also showed 30-70% lower AMP induced DA_{rel} than controls, though at a trend level in a study of 10 alcoholics and 8 controls. In a study of 8 patients with schizophrenia, DArel was induced by both amphetamine and low specific activity raclopride (LSA RAC) and resulted in large changes in the inhibition (X3) due to phasic DA changes: 50% in X3 after AMP, and >100% change in X3 after LSA RAC. Finally, in a preliminary study of two patients with RLS and 4 age-matched controls, there is a 50-100% increase in DA_{rel}, although only a trend level with these small numbers. This wide range of ongoing research shows that PET/SPECT methodology can detect the full range of DA_{rel} changes that maybe seen in psychiatric and neurological conditions. These results will be discussed to illustrate the contributions and progress made in the study of in vivo dopamine psychostimulant perturbation studies.

PET Imaging of Extrastriatal Dopamine Release and Baseline Levels of Extracellular Dopamine

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While striatal dopaminergic neurotransmission is likely involved in a number of disorders including schizophrenia, Tourette's syndrome, attention deficit disorder, depression, and drug abuse, there is considerable data in humans and animals indicating the role of dopaminergic neurotransmission in cortical and limbic regions, the thalamus, and the substantia nigra in these disorders. There have been some studies of dopamine (DA) D2 and D1 receptors in extrastriatal regions, but there has been a lack of methods for assessing phasic and tonic dopamine release in regions outside the striatum. Studies of d-amphetamine induced DA release in humans using PET with [18F]fallypride performed prior to and following oral d-amphetamine administration will be presented; the results demonstrate that these studies can be used to estimate phasic DA release in the striatum, substantia nigra, amygdala, and cortical regions. The effects of d-amphetamine induced DA release in the caudate and putamen on [18F]fallypride binding potentials are similar to those seen with [11C]raclopride while the effects in extrastriatal regions are less but still significant. The correlation of d-amphetamine induced DA release with changes in cognitive function and affect will be discussed. Alphamethylparatyrosine depletion of cerebral DA in conjunction with imaging of striatal DA D2 receptors has provided a method for estimating baseline, i.e. tonic, levels of extracellular DA in striatum. Initial results from[18F]fallypride PET studies performed prior to and following alphamethylparatyrosine induced depletion of cerebral DA to assess baseline, extracellular levels of DA in extrastriatal regions will be presented, Attempts to image cognitive activation of DA release in extrastriatal regions will be reviewed. The results of these studies will be integrated with current knowledge of the roles of synaptic and volume modes of neurotransmission in striatal and extrastriatal regions. The application of these methods to the study of cognitive function and disorders believed to be mediated by abnormal phasic and/or tonic DA release in extrastriatal regions will be discussed.

Role of NMDA and GABAB Receptors in Cortical and Subcortical Dopaminergic Regulation

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Schizophrenia is associated with hyperactivity of amphetamine (AMPH)-induced dopamine (DA) release that is particularly associated with the decompensated phase of the illness. Although mechanisms underlying DA dysregulation are unknown, similar deficits can be induced in both humans and rodents by administration of NMDA antagonists, suggesting that NMDA dysfunction may contribute. The present studies investigate mechanisms underlying NMDA receptors in regulation of AMPH-induced DA release in prefrontal cortex and striatum using in vitro release assays and in vivo microdialysis. Subchronic treatment with PCP led to a significant potentiation of AMPH-induced DA release that was reversed by administration either of the NMDA glycine-site agonist glycine, or glycine transport inhibitors including NFPS, NPS1000 or Org25935. Effects of NMDA glycine-site agonists were apparently mediated by stimulation of local GABA release, which inhibited presynaptic DA release acting at a GABAB receptor. Subsequent studies with GABAB agonists and antagonists have confirmed the ability of GABAB receptors to regulate DA release in both striatum and PFC, suggesting a potential site for intervention in disorders, such as schizophrenia and methamphetamine/cocaine abuse that are associated with DA hyperactivity. Further, differential effects of specific GABAB ligands suggest differential roles of specific GABAB receptor subtypes. Overall, these findings highlight the role of NMDA receptors on GABAergic interneurons in DA regulation, and suggest appropriate sites for therapeutic intervention.

Effect of Acute Systemic Baclofen on Amphetamine Stimulated Dopamine Release as Measured in Rats Brain with the Dopamine Receptor Tracers [^{3}H]Raclopride and [^{3}H]-(+)-PHNO

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The $GABA_B$ receptor agonist baclofen attenuates the reinforcing effects of cocaine and amphetamine in rats, and appears to blunt craving in patients addicted to cocaine. With respect to mechanism of action, microdialysis studies in rats have revealed that baclofen attenuates psychostimulant induced increases in dopamine efflux in the nucleus accumbens. In schizophrenia, an illness characterized by increased amphetamine stimulated dopamine release, a reduction of $GABA_B$ receptor densities in the frontal cortex and other regions has been observed post-mortem. This is the first study that uses radiotracer methods to monitor the effects of $GABA_B$ receptor agonists and antagonists on amphetamine stimulated dopamine release in the striatum and other brain regions in rodents. The two radiotracers

used were the D_2/D_3 receptor antagonist tracer, [^{3}H]raclopride, and the D_3 receptor selective agonist [^{3}H]-(+)-PHNO. We first demonstrated that baclofen alone does not alter the binding of either tracer in the striatum. However, different sensitivities were noted between the two tracers in measuring baclofen's effect on amphetamine induced dopamine release ([^{3}H]raclopride was more sensitive than [^{3}H]-(+)-PHNO). We hypothesize that this might be due to different dopamine receptor binding characteristics of the two tracers in the intracellular environment, or a reduction in the degree of G-protein coupling upon amphetamine challenge that [^{3}H]raclopride is insensitive to. In addition, reduced [^{3}H](+)-PHNO binding in the thalamus was found in baclofen treated animals compared with controls. This is a novel finding that will be discussed in some detail. Taken together, these data support that $GABA_B$ receptor activation by baclofen attenuates amphetamine stimulated striatal dopamine release, and that this subsequently impacts occupancy of dopamine at $D_2/_3$ receptors. In addition, the data reveal that specific dopamine receptor PET tracers may be used to follow the effects of $GABA_B$ agonists on psychostimulant drug induced dopamine release in animal models and humans. In addition to drug addiction research, these findings may be relevant to the study of schizophrenia, where the reported loss of $GABA_B$ receptors in the CNS may be at least partly responsible for the well-documented sensitivity to amphetamine in these patients.

Panel Session
Treatment of Frontotemporal Dementia: Identifying Pathophysiologic Targets

Clinicopathologic Analysis of Ubiquitin and Tau in FTD

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This presentation will examine the relationship between early clinical features, pathologies, and biochemistry of the frontotemporal lobar degenerations (FTLDs). Pathologic reexamination with the most recent immunohistochemistry of all cases diagnosed with FTLD, PSP, and CBD between 1970 and 2004 revealed that ubiquitinated inclusions are at least as commonly seen as tau-positive inclusions. All cases of FTD-MND were tau-negative and had pathologic evidence of motor neuron degeneration. All cases classified as PSP-like or CBD-like had tau-positive pathology. Of the 13 cases with PNFA, PSP and CBD accounted for almost 70% of the cases, while FTD was almost equally divided between tau-positive and tau-negative diseases. Dr. Josephs will bring these findings to bear on the strategization of future interventions for FTD.

The Role of CSF Biomarkers in the Diagnosis of Frontotemporal Dementia

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Earlier studies on candidate biomarkers in cerebrospinal fluid (CSF) for frontotemporal dementia (FTD) such as total tau, hyperphosphorylated tau, the 42 amino acid fragment of b-amyloid, S100b and neurofilament will be reviewed. To discover novel possible biomarkers for FTD we analysed CSF samples from FTD patients and controls by surface enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry (MS). Sixteen clinically diagnosed FTD patients and 12 non-demented controls were included in the study. The samples were analysed on four different array surfaces using two different energy-absorbing molecules as matrices. In total each CSF sample was subjected to eight different surface/matrix conditions. About 2000 protein peaks (mass/charge ratios) were detected. Forty-two peaks were differentially expressed in FTD ($p < 0.01$). After exclusion of peaks with low signal-to-noise ratio and peaks representing differentially charged proteins, 10 peaks remained, five of which were overexpressed and five underexpressed in FTD cases compared to controls. The combination of these biomarkers discriminated FTD from non-demented controls almost completely. Six of the peaks were purified further and identified by tandem MS.

Frontal-Subcortical Circuits and a Rationale for Neuroreceptor Quantification in FTD

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The clinical manifestations of frontotemporal dementia (FTD) correspond to symptoms of frontal-subcortical circuit (FSC) syndromes. The constellations of impairments in FTD include changes in personality, social behavior and motivation. Cognitive losses in FTD follow the pattern of the dorsolateral prefrontal dysexecutive syndrome, affecting attention, planning, and problem solving. Neuropsychiatric symptoms include depression, anxiety, impulsivity and compulsive behaviors. Lesions to the dorsolateral prefrontal FSC can result in depression in addition to the difficulties with goal-directed activities and self-monitoring. Degeneration of the orbitofrontal FSC results in problems with social behavior and affect, such as irritability, loss of empathy, and obsessive-compulsive behaviors. Lesions of the superior medial frontal FSC lead to amotivational syndromes, such as apathy. Function of the FSCs is subject to the modulation of the striatum by dopamine (DA) and serotonin (5-HT). DA receptors (DR) play important roles throughout the basic FSC model, one of which is to complete a self-regulatory DA secretion loop involving the anterior cingulate and substantia nigra pars compacta. In addition, the connectivity of the subcortical aspects of any FSC runs through D1R receptor-laden striasomal components and D2R-heavy matrix. The net effect of these influences on the phosphorylation of DARPP-32 (a phosphoprotein) modulates the activity of the FSC. The development of parkinsonism in later stages of FTD implies that at least a loss of dopaminergic tone in the motor FSCs is part of the illness, but associations of DA with motivation, mood, and frontal executive function are likely to impact the clinical manifestations of FTD as well. The basal ganglia and thalamus are atrophied even early in FTD, indicating the potential for derangements in DR densities. Serotonin may be linked to FSC syndromes and therefore FTD by dint of its effects upon the DA system (5-HT3R) or because of localization to limbic (5-HT1a- and 2aR) and prefrontal cortices (5HT1d- and 2aR). Radioligand PET imaging allows us to quantify DR and 5-HTR in vivo. With FSC syndromes as the theoretical model for the pathophysiology of FTD, examination of DR and 5-HTR in living patients with FTD may elucidate targets for pharmacotherapy. Dysfunctional attitudes in depression outside the context of FTD are linked to a relative increase in 5-HT2aR binding. Preliminary 5HTR studies on subjects with FTD have shown reductions in both 5-HT1a- and 2aR in frontal cortex, but DR imaging may reveal subcortical aspects of FTD pathology.

Pharmacotherapy in Frontal Variant Frontotemporal Dementia

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The frontal variant of frontotemporal dementia (fvFTD) typically occurs at a younger age than most dementias when patients still have parental and financial responsibilities for children. Despite being one of the most prevalent forms of early-onset dementia, there currently exist few treatments to improve the cognitive and behavioural deficits, in contrast with the management options for dementia of the Alzheimer type. Patients with fvFTD present with risky decision-making behaviour, impulsiveness, disinhibition, apathy, altered appetite and stereotypic behaviour. We originally proposed that the or-

bitofrontal cortex was a major locus of aberrant function in fvFTD. This was based on objective neurocognitive assessment indicating risky decision-making and deficits in reversal learning, which resemble the cognitive sequelae of patients with damage to orbitofrontal cortex. These reward-based deficits are considered to be one of the main problems that fvFTD patients face, in contrast to the early episodic memory dysfunction of patients with dementia of the Alzheimer type. Subsequent post mortem and in vivo neuroimaging studies by other research groups have established neuroanatomical evidence for orbitofrontal dysfunction in fvFTD. Given the roles of dopamine, noradrenaline and serotonin in the modulation of risk-taking, decision-making, impulsive behaviour, reward-based learning and mood, we assessed the potential cognitive enhancing effects of methylphenidate and paroxetine in fvFTD. The results of these studies together with suggestions for future studies using other cognitive enhancers, such as modafinil and atomoxetine, will be discussed. Acknowledgements – This research was funded by the Wellcome Trust and Medical Research Council (UK). Declaration – BJS consults for Cambridge Cognition Ltd.

Panel Session
VMAT2 in Health and Disease: Individual Differences, Imaging and New Therapeutics

VMAT2: Imprinted Allelic Variation and Roles in Narcolepsy, Vulnerability to Amphetamine Dependence and Tetrabenazine Responses

Tomas Drgon*

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Studies in VMAT2 heterozygote knockout mice have defined the plieotropic effects that differential levels of expression of this gene are likely to exert on a number of drug related and physiological phenotypes in humans. I present data from 1) transmission of VMAT2 haplotypes in narcolepsy that defines significant TDT with strong maternal-parent of origin influences, 2) identification of the VMAT2 promoter region sites that are methylated in ways that are likely to account for this parent of origin effect and in ways that correlate with the levels of VMAT2 expression in postmortem samples, 3) development of assays that monitor both VMAT2 haplotype and methylation in unrelated individuals, 4) use of these assays to identify links between these imprimted VMAT2 haplotypes and vulnerability to methamphetamine dependence and 4) use of these assays to define differential responses to tetrabenazine in subjects from the TETRAD HD trial that relate to VMAT2 haplotype. Taken together with kkockout mouse data, these human results strongly support the usefulness of assessing common imprinted VMAT2 haplotypes in a variety of pharmacological and pathological settings.

Tetrabenazine for Huntington's Chorea

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Chorea contributes significantly to the burden of disability experienced by Huntington's disease patients. Tetrabenazine (TBZ) is a potent physiologic VMAT2 antagonist that has been successful in treating chorea in open-label trials, but had not been subjected to randomized, double blinded, controlled clinical trials. We randomized 84 ambulatory patients with HD to receive TBZ or placebo. An increasing dose regimen stopped at 1) 100 mg/day, 2) when the desired antichoreic effect occurred or 3) when adverse effects supervened. With this regimen, TBZ reduced chorea evaluation scores by 5 while placebo treated patients reduced scores only 1.5. This adjusted mean effect size of -3.5 +/- 0.8 units was significant, as were assessments of global clinical global improvement. I will describe the adverse events identified in this study, the results of follow up of these patients, and our conclusions about the efficacies of TBZ and the need to individualize its dosages to effectively treat chorea in Huntington disease while minimizing side effects (for the Huntington's Disease).

PET Imaging of the Vesicular Monoamine Transporter Type-2 in Neurodegenerative and Neurobehavioral Disorders

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The type-2 vesicular monoamine transporter (VMAT2) is an essential molecular component of presynaptic nerve terminals that release dopamine, serotonin, norepinepherine, epinephrine or histamine. Our laboratories have developed methods for mapping, quantifying and in vivo imaging of VMAT2. Preclinical research employing quantitative in vitro VMAT2 autoradiography indicates that striatal binding sites for tetrabenazine derivative ligands specifically identify VMAT2 expression. Levels of striatal VMAT2 binding are attributable almost entirely to presence of nigrostriatal dopamine projection terminals. Striatal VMAT2 binding sites are not readily regulated by pharmacological treatments that affect dopamine neurotransmission, and are closely related to the integrity of substantia nigra dopaminergic cell bodies in neurotoxin lesions. Clinical research quantification of VMAT2 is accomplished employing the PET radiotracer [11C]dihydrotetrabenazine (DTBZ) – the active metabolite of the orally-active VMAT2 inhibitor tetrabenazine. After intravenous injection of [11C]DTBZ, PET imaging and mathematical modeling permit quantification of VMAT2 binding sites in the striatum, diencephalon and brainstem. DTBZ has 2 isomers, and we have identified that the pharmacological activity and specific brain VMAT2 binding resides with (+)DTBZ. Contrasting [11C](+)DTBZ and [11C](-)DTBZ distributions indicates that the occipital cerebral cortex may serve as a reference region, devoid of specific binding, for use in model-based estimations of specific (+)[11C]DTBZ binding. Clinical investigations of (+)[11C]DTBZ binding reveal changes in a number of conditions. In Parkinson disease (PD), the expected losses of striatal VMAT2 binding sites are observed. Striatal binding reductions are asymmetrical, affect the dorsal striatum (posterior putamen) most severely, and correlate significantly with clinical measures of disease duration and distribution of limb rigidity and bradykinesia. There is demonstration of considerable preclinical losses of striatal VMAT2 binding, with an average reduction of 68% of total striatal and 73% of posterior putamen binding prior to emergence of clinical abnormalities in the unaffected hemispheres of hemi-parkinsonian subjects. Reductions of VMAT2 binding sites are observed also in the striata of subjects with remote prior exposure to recreational psychostimulants. In this instance, striatal binding reductions are more uniform, and of slightly greater intensity in the caudate nucleus. Overall, striatal binding is reduced by 10% in prior methamphetamine abusers, arguing for a neurotoxic effect, but one that is well below the threshold for manifesting abnormal control of movement as revealed in PD. Finally, we have identified an increased level of VMAT2 expression in the ventral striatum of subjects with Tourette syndrome, approximating the anatomic location of the nucleus accumbens septi. This suggests the possibility that an increased level of presynaptic dopamine terminals may account for some of the clinical features of TS and for therapeutic effects of treatments targeting dopamine neurotransmission. The involvement of VMAT2 expression in neuropsychiatric disorders and the anticipated availability of clinical pharmacological approaches to reversible VMAT2 inhibition make this molecular target of particular current interest.

Tetrabenazine is Safe and Effective for the Treatment of Hyperkinetic Movement Disorders

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Tetrabenazine (TBZ), a dopamine-depleting agent first synthesized in the 1950s, was initially developed for the treatment of schizophrenia. Since 1979 when an IND for compassionate use of TBZ was granted

to J. Jankovic, MD, Baylor College of Medicine, Houston, Texas, well over 1,000 patients with hyperkinetic movement disorders were treated at Baylor with TBZ. We report here the results in more than 500 patients for whom we have adequate follow-up. A simple response rating from 1 = marked improvement to 4 = no response to 5 = worsening was assessed initially and at the last clinic visit for TBZ treatments that lasted an average of almost 30 mos. Global response ratings indicating marked improvement were recorded in the vast majority of patients with tardive stereotypy, myoclonus, Huntington's disease and tardive dystonia. Patients with Tourette's syndrome and dystonia also benefited. The most common averse effects, occurring in about 20% of all patients, included drowsiness, parkinsonian depression, insomnia, anxiety, and akathisia. Side effects were controlled with dosage reduction. We conclude that TBZ is an effective and safe drug for the treatment of a variety of hyperkinetic movement disorder in most individuals.

Thursday, December 7, 2006

Panel Session
Evaluating the Efficacy and Safety of Antidepressants for Depression and Suicide Risk in Youth and Adults

Efficacy and Safety of Antidepressants for Depression and Suicide Risk in Youth and Adults – Results of New Analyses

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Recent papers that have studied the relationship between completed suicide and SSRI use at an ecological level, have shown that increased SSRI use leads to decreased suicide in both adult (Gibbons, et.al. 2005), adolescents (Olfson et.al., 2003). These results are at odds with FDA meta-analyses of randomized clinical trials (RCTs) that show increased risk of suicidal ideation in children treated with SSRIs. We present the results of several new analyses of different datasets across the lifespan that shed further light on the risks and benefits of SSRIs.
Gibbons R, Hur K, Bhaumik D, Mann J: The relationship between antidepressant medication use and rate of suicide. Archives of General Psychiatry 2005; 62:165-172. Olfson M, Shaffer D., Marcus SC, Greenberg T: Relationship between antidepressant medication treatment and suicide in adolescents. Archives of General Psychiatry 2003;60:978-982.

Effects of Psychotropic Drug Treatments on Suicide Risk

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Background: Major psychiatric disorders are associated with increased mortality, largely accounted for by greatly increased risk of suicide in mood and psychotic disorders. Until remarkably recently, empirical research on effects of psychotropic treatments on suicide risk has been lacking. Based largely on a rare, randomized, controlled trial (InterSePT) of clozapine vs. olanzapine with suicidal behavior as an explicit outcome measure, clozapine is the only treatment with an FDA-approved indication for reducing suicidal risk.
Methods: There now are sufficient data to support meta-analyses of treatment effects on suicide. We have carried out updated analyses for: [a] clozapine in schizophrenia, [b] lithium in bipolar disorder, [c] lithium in recurrent unipolar major depression, and [d] antidepressants in major depression, based on computerized literature searches, adding new data, and using standard statistical models.
Results: There is substantial evidence of an antisuicide effect of clozapine in schizophrenia, at least at the level of attempts. Evidence for reducing risk of both suicides and attempts, the intensity of suicidal ideation, as well as the lethality of suicidal acts (increased attempt/suicide ratio) with lithium treatment in bipolar disorder is consistent and strong, including data from randomized, controlled trials. Benefits of anticonvulsant mood-stabilizers appear to be inferior to lithium. Emerging evidence suggests similar benefits of lithium in unipolar recurrent major depression. Antidepressants do not reduce risk of suicides or attempts but are associated with reduced suicidal ideation in randomized, placebo-controlled trials in adult depression. Analyses of antidepressant trials in children indicate limited therapeutic benefits and statistical non-separation of SSRIs from TCAs, with evidence of greater mood-destabilizing effects in young children exposed to SSRIs and in adults treated with TCAs, all to be balanced against suggestions of increased risk of suicide attempts in children.
Conclusions: Controlled trials in which suicidal behaviors are explicit outcome measure remain rare. Nevertheless, available evidence indicates substantial antisuicidal benefits of several classes of psychotropics. These findings strongly encourage further empirical research on mortality risks, more broadly, if ethical and feasible methods can be devised. [Supported, in part by grants from the Bruce J. Anderson Foundation, JDS Corporation, and by the McLean Private Donors Psychopharmacology Research Fund.]

Development of the Serotonergic System: Implication for Mood Disorders

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There are dramatic developmental changes in the brain in the serotonergic system during development. Serotonin has been found to be altered in various psychiatric disorders, including mood disorders and suicide. Many effective therapeutic agents target various serotonergic receptors, including the serotonin transporter (SERT, SSRIs antidepressants) and 5-HT1A receptor antagonists (Anxiolytics). We previously reported increased 5-HT1A binding in the ventrolateral prefrontal cortex of suicide victims. We compared 5-HT1A binding in the prefrontal cortex (PFC) of suicides (alcoholic and nonalcoholic), alcoholic nonsuicides and normal controls with negative toxicological screens for other drugs (n=118). The nonalcoholic suicide group has approximately 20% more binding than controls in ventrolateral PFC, whereas the alcoholic groups have 20% less 5-HT1A binding than controls in the same region. Binding correlated with age, thus we dichotomized the sample into adult (>25y) and youth (≤25y) groups and reexamined the comparisons. In the adult group (n=82) suicides have more binding in the lateral PFC and decreased binding in the orbital cortex. In the young group (n=36) nonalcoholic suicide victims have more binding than controls in lateral, medial and orbital PFC. Thus, alterations in 5-HT1A binding are much more striking and widespread in young suicide victims than in adults. Work by others (Pandey et al., 2003) indicates that the increase in 5-HT2A receptors in suicide, is more pronounced in adolescents than in adults. Despite the presence of a large literature suggesting that there is a deficit in serotonergic neurotransmission in mood disorders and suicide, we have evidence that depressed suicides have more serotonin neurons in the dorsal raphe nucleus (DRN) and have more neuronal tryptophan hydroxylase (TPH2) protein and TPH2 mRNA. In agreement with the reduced levels of SERT in many brain regions, identified by in vivo PET and in postmortem studies, there are fewer DRN neurons expressing SERT mRNA, but those who are expressing it, are overexpressing SERT mRNA. Transiently blocking the SERT sites in the mouse early in development with SSRIs appears to affect brain circuits involved with the stress response, permanently affecting emotional and stress-related behaviors in adulthood (Ansorge et al., 2004), with the SSRI mice resembling mice that lack the SERT gene. Individuals with one or two copies of the short SERT allele are more susceptible to mood disorders and suicide if they were subjected to

childhood adverse events (Caspi et al., 2003), implicating gene-environment interactions. In vivo PET work in our group (Parsey et al.,2006) suggests that the SERT promoter variants do not affect SERT binding in brain in healthy or depressed adults, but depressed patients have less SERT binding than controls. These results are in agreement with recent findings by Caspi (2003), suggesting the need of involving childhood or adolescent stressors to effect a change later in life. Our in vitro work (Mann et al., 2000) did not show a correlation between SERT promoter variants and binding by autoradiography, as it was shown by Lesch et al., (1996), and we also showed a generalized reduction in SERT binding in depression. Marked changes in the serotonergic system during development are increasingly reported, suggesting that serotonergic markers do not become "stable" until adulthood. This neurodevelopmental aspect of the serotonergic system may be particularly affected in mood disorders with more pronounced effects in youthful populations, something to consider when trying to understand the effects of drugs that target the serotonergic system.

Combining Data and Clinical Expertise for Algorithmic Prediction of Suicide Attempts

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Background: A computerized decision rule for predicting suicide attempts would increase the chances of preventing them and therefore, e.g., make it possible for more suicidal patients safely to take part in clinical trials. A decision rule cannot take the place of clinical judgement, but would provide useful supplementary information. We use new statistical methods to compute attempt prevention decision rules. As a practical matter, prediction of attempt means recommending in a timely fashion the needed level of preventive care. We have prototyped a statistical algorithm that incorporates clinical expertise and data on suicide attempts to generate decision rules. These rules recommend treatment level based on patient information. The algorithm makes use of cost benefit analyses for attempt prevention that include all costs, broadly considered, of attempts and preventive treatment. Clinicians can help formulate cost benefit analyses. Even a rough cost benefit analysis is useful. Our algorithm "learns" decision rules from data on suicide attempt and predictors ("training data"). The algorithm's design uses ideas from "machine learning" (ML), a new trend in statistics and artificial intelligence research.
Methods: We estimate the expected cost ("risk") of a candidate decision rule from data. Our algorithm searches for the decision rule that minimizes the estimated risk. There are literally infinitely many "terms" in our models, but we reduce model complexity using standard ML methods. We tried our method on data on N=304 patients. Patients were assessed at baseline and at approximately 3, 12, and 24 months. 52 of the 304 made follow-up attempts. Only baseline predictors were used and we only tried to predict the first follow-up attempt. The predictor variables were age, sex, unipolar or bipolar diagnosis, presence of co-morbid personality disorders, history of past suicide attempts, smoking, recent life events as measured on the 7-point Ramsey scale, objective depression score as measured by the Hamilton scale, scores on the Beck Depression Inventory, Beck Hopelessness Scale, Scale for Suicidal Ideation, Reasons for Living Inventory, Brown Goodwin Aggression Scale, Buss Durkee Hostility Scale, Barratt Impulsivity Scale, and Global Assessment Scale (not considering suicidality). To illustrate our method, for a cost benefit analysis we chose 20,000 (in arbitrary units) as the cost of an attempt. We assumed that as treatment level increases the probability of an attempt drops off exponentially, with a treatment level costing 2 units needed to cut the probability in half.
Results: These results are preliminary. A decision rule that always makes the same recommendation, i.e., is constant, is useless. Therefore, the amount of variability, across time and subjects, in the recommended treatment level is a measure of how much information the modeling algorithm finds in the training data. Our fitted decision rule recommended treatment intensities that correspond to reductions of from 0 to 89% in attempt probability with first and third quartiles of 22% and 67%, respectively. Thus, there is much information in these training data.
Discussion: These preliminary results suggest the feasibility of constructing algorithms for prediction of attempts. We have only considered the problem of predicting the first attempt after baseline. Extending the idea to multiple attempts is straightforward. Developing computerized attempt prediction rules also includes constructing cost benefit analyses and designing a clinical trial to test the method in practice.

Panel Session
Integrative Genomics of Alcoholism

The Origins and Consequences of Variation in the Human Alcohol Response

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Background: This presentation focuses on recent developments regarding deep sequencing of candidate genes in the UCSD/UCSF Gallo Center sib-pair study searching for genes related to the low level of response (LR) to alcohol as a risk factor for alcoholism. A low LR is an established endophenotype related to the alcoholism risk as it is seen before the disorder develops, predicts an enhanced risk for alcohol use disorders (AUDs), and is genetically influenced with a heritability as high as 60%. Prior linkage and association studies using both alcohol challenges or a retrospective self-report questionnaire to measure LR in humans as well as evaluations of the alcohol response in animals have highlighted chromosomal regions (e.g., on chromosome 10) and specific candidates (e.g., the GABA cluster on chromosome 5 and the l allele of the serotonin transporter) potentially related to a low LR.
Methods: We have identified 350 sib-pairs who were drinking but not alcohol dependent 18-to-25 year-old children of alcoholics. Currently, the number of sib-pairs with available phenotypic data (alcohol challenge-based LR) and genotyping have increased, offering the opportunity to focus on deep sequencing of candidate genes of interest.
Results: The chief finding is that many of the candidate sequence variants involve relatively rare (e.g., <5% of the population) alleles. Data will be offered on the possible relationship to LR of variants of several specific genes, including components of GABA clusters, genes on chromosome 10 such as CYP2E1 and KCNMA1, polymorphisms for OPMR1, and several components of the intracellular signaling system such as variations in adenylate cyclase and the signal transduction-related gene DGKz.
Conclusion: Due to rarity of many of the candidate alleles identified by deep sequencing, the UCSD/UCSF collaboration has now initiated the collection of DNAs and retrospective self-report-based LR's from relatives of the original sib-pairs who themselves demonstrated a gene variant of interest. This presentation will discuss specific findings from the deep sequencing phase of the sib-pair analyses, as well as the family extension results.

Gene Identification in the Collaborative Study on the Genetics of Alcoholism Project: Finding Genes and Characterizing the Spectrum of Risk

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Background: Alcohol dependence is a complex genetic disease, with substantial heterogeneity. Alcohol dependent individuals often meet criteria for other psychiatric disorders, including depression. The Collaborative Study on the Genetics of Alcoholism (COGA) has

identified several genes involved in the predisposition toward alcohol dependence, and is further exploring the relationships with other conditions.
Methods: The strategy is to conduct family-based linkage analyses to identify regions containing genes that influence the phenotype, followed by association analyses of candidate genes within the linked regions to identify specific genes in which variations affect risk for alcoholism or other phenotypes. We have examined clinical phenotypes including alcoholism and depression and have also examined neurophysiological and other endophenotypes. Association analyses have used multiple SNPs within each candidate gene, in an attempt to cover a large fraction of the genetic variability. Given our sample, our primary analyses have been family-based association studies (e.g. Pedigree Disequilibrium Test).
Results: The strategy of moving from linkage to association has been successful, with the identification of several specific genes in which variations affect risk for alcoholism, including GABRA2 (Edenberg et al., 2004), GABRG3 (Dick et al., 2003), CHRM2, (Wang et al., 2004), TAS2R16 (Hinrichs et al., 2005) and ADH4 (Edenberg et al., 2006). The use of endophenotypes has assisted in our identification of these genes. Efforts are currently underway to better characterize the risk associated with these genes. Some are associated with other psychiatric phenotypes, including depression. Some are associated with abuse or dependence on illicit drugs, which may be a reflection of a more severe form of the disease. In one case, we have found an association with conduct disorder symptoms in adolescents and alcoholism in adults.
Discussion: The use of endophenotypes has aided our identification of genes in which variations affect risk for alcoholism. Further exploration of the effects of these genes on comorbid conditions and during the life cycle has led to interesting findings, showing shared genetic risk factors. The extensive characterization of subjects provides a rich database for exploring these connections to better understand alcoholism and related conditions.

A Genetic/Genomic Analysis of Alcohol Intake: The Use of MAGIC-B

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Background: Translational research in studies of alcoholism is beginning to bear fruit. Preference for alcohol, measured as voluntary alcohol consumption by rodents, represents an endophenotype thought to be related to susceptibility to alcohol dependence in humans. The selective breeding of lines of rats and mice that differ in alcohol preference demonstrates a genetic influence on this trait and studies with humans have also demonstrated a genetic influence on the quality of ethanol consumed buy individuals. Some of these genetic influences relate to individual differences in alcohol metabolism, and some of the genetic differences related to brain mechanisms by which ethanol produces pleasurable or aversive effects. Genetic differences can arise from functional polymorphisms in the coding regions of genes or in the control regions responsible for transcriptional events.
Methods: Novel methods have been developed to ascertain, simultaneously, the expression levels for thousands of genes in brain and other organs. We will describe the application of the novel gene array data analytical technique, "MAGIC-B" (microarray ascertainment of genes that influence complex behaviors) to identify candidate genes associated with alcohol preference. Gene expression profiles in brains of two replicate lines of mice selectively bred for high alcohol preference (HAP mice) or low alcohol preference (LAP mice) were determined with Affymetrix mouse whole genome oligonucleotide arrays, and differential gene expression between HAP and LAP mice was determined with statistical analysis. The chromosomal localization of the regulation of expression of the differentially expressed genes was determined with quantitative trait locus (QTL) analysis. To find the genomic regions for control of gene expression (cis or trans), a database of gene expression in brains of animal from thirty BXD RI mouse strain was generated and used to ascertain expression QTLs.
Results: Genes with expression QTLs (eQTLs) that overlapped with QTLs previously determined to be associated with alcohol preference (behavioral QTLs) were identified as candidate genes for alcohol preference. These genes can be organized into signal transduction pathways that include signaling systems (cyclic AMP/PKA, PKC) previously associated with alcohol preference, as well as pathways involved in neuronal survival and differentiation, that may also be related to neuroadaptation to alcohol. To provide further confirmation of the importance of some of the identified genes in alcohol preference, the expression levels of the genes were correlated with drinking behavior in eight strains of BXD RI mice and the parental C57Bl and DBA mice. As an example, the expression levels of Gnb1 (a G protein β subunit) and HYOU (an endoplasmic Ca++ buffering protein) were highly correlated (r2=0.9 and 0.92 respectively) with measures of alcohol preference.
Discussion: The biological implications of the differentially expressed gene product and relations with QTL studies with humans will be demonstrated. (NIAAA and Banbury Fund)

Accessing Addictions Neurobiologies with a 130 gene, 1536 Marker Array

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Background: Addictions heritabilities range from moderate to high, with a surprising correlation between addictive liability and heritability indicating the importance of addictions-shared neurobiologies such as stress/anxiety responses, behavioral control, reward, and obsessionality. Human and mouse ethanol QTLs implicate GABAA gene clusters and the serotonin transporter, both of which may have roles in anxiety. Both are also repesented on an integrative genetics tool we have developed: a genotyping array for genetic epidemiology of addictions and other psychiatric diseases.
Methods: We assembled a 1536 SNP (single-nucleotide polymorphism), 130 gene array that includes 186 ancestry informative markers (AIMs) with allele frequencies defined in Hapmap. The AIMs were selected to differ in allele frequency by at least 0.7, and ten-fold, between at least two ethnic populations. The array is implemented as an Illumina Goldengate assay, and largely focuses on genes implicated in shared addictions neurobiologies, using SNPtagging to capture all non-rare haplotypes. The array includes loci that alter function. Furthermore, haplotypes predict expression of certain genes such as COMT and TPH2 and, as shown here using differential allele expression, HTT. We applied this array to alcoholism/psychiatric disease datasets, each with >400 psychiatrically interviewed subjects, including Finns, Southwest Indians and Plains Indians . Candidate gene linkages to be presented include GABAAR2 and HTT.
Results: Genotyping of ethnically well-defined populations: Finns, Plains Indians, Han Chinese and African Americans validated marker allele frequencies, between population differences in allele frequencies, and linkage disequilibrium relationships predicted by HapMap. The AIMs enabled quantitation of ethnic admixture of individual subjects. We followed up alcoholism linkage to the Chr 4 GABAA receptor gene region, where two groups reported an alpha 2 haplotype association. In American Indian and Finnish samples, we found the same GABRA2 haplotype is involved as previously reported but furthermore this association is anxiety-mediated, as shown using the median split for Harm Avoidance. We had reported that serotonin transporter gain-of-function alleles at two separate loci are linked to OCD. These findings were made in two families carrying a particular rare variant (Ile425Val), in a large OCD case/control dataset (NIMH), and in affected child/parent

trios (Clarke Institute). Here we use haplotype-based analysis to identify a new, independent functional HTT locus in the 3'region. This locus shows linkage to OCD in both the case/control datset and the parent-child trios, with gain-of-function haplotype again implicated.
Discussion: Replication of Hapmap linkage disequilibrium relationships for the SNPs on the genotyping array strongly implies that haplotype coverage will be as anticipated using haplotype tagging . Genotyping arrays such as this one enable the systematic exploration of genes implicated in addictions neurobiologies. Regarding particular genes on the array, human and mouse QTLs indicating the involvement of GABAA subunit gene clusters in alcohol-related behaviors have now been followed up by three groups to the level of a specific haplotype at the alpha2 gene. We find that this haplotype exerts its effect via the anxiety dimension. Multiple HTT functional variants impact connectivity between brain regions and thereby alter behavioral dimensions, including anxiety and obsessionality, that are relevant to addictions neurobiology.

Panel Session
Mechanisms of Stress-Induced Modulation of Prefrontal Cortex Circuitry and Function

Chronic Stress Alters Pyramidal Cell Morphology in Medial Prefrontal Cortex

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Background: Chronic stress can impact future behavioral responses, suggesting that it leads to long-term alterations in cortical circuitry. Early studies emphasized morphologic alterations in hippocampus and amygdala that might underlie such behavioral effects. However, given that stress-induced behavioral alterations involve the cognitive realm of prefrontal cortex, we hypothesized that alterations in prefrontal cortical circuitry might also contribute directly to the long-term behavioral consequences of stress.
Methods: Rats were either in control groups or exposed to 10 or 21 days of chronic stress, and then perfused and pyramidal cells in layer III of medial prefrontal cortex (mPFC) of both groups were injected with Lucifer yellow. The cells that satisfy stated criteria for inclusion are then reconstructed and quantitative analyses of dendritic arbor, complexity and spine density are done to determine the effects of stress. We have targeted and analyzed pyramidal neurons in all three areas of rat mPFC: anterior cingulate (AC), prelimbic (PL), and infralimbic (IL).
Results: Chronic stress leads to dendritic retraction in AC, PL, and IL. We have also completed an analysis of spine density in AC/PL and demonstrated a stress-induced loss of 20% of the spines on the same layer III neurons, leading to a total spine loss of 33%. In addition, if the animals are given 3 weeks to recover from the chronic stress, such morphologic alterations are no longer apparent, suggesting that these effects are reversible. Finally, recent studies of ovariectomized female rats suggest that these effects may be circuit-specific and also sensitive to estrogen levels.
Conclusion: Prefrontal cortex mediates cortical processes of the highest order such as cognitive and motor planning. Chronic stress has the potential to alter future behavioral responses dependent on the cognitive processes mediated by prefrontal cortex. These findings provide a morphologic and circuitry-based substrate for such long-term alterations, and also demonstrate that they are reversible. Present and future studies are to reveal which prefrontal circuits and neuron classes are most sensitive to stress, as well as the influences of gender and estrogen levels on stress-induced morphologic alterations of mPFC.

Chronic Stress Induces Functional Trafficking of the Norepinephrine Transporter in the Prefrontal Cortex

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Background: The critical cognitive and behavioral functions of the prefrontal cortex (PFC) require extensive modulatory control by brainstem monoamines. The norepinephrine (NE) input from the locus coeruleus (LC) to the PFC facilitates these functions under conditions of arousal, vigilance and selective attention. NE transmission is dynamically regulated by many factors, including cell firing, enzymatic synthesis, and reuptake by plasma membrane transporters. We have shown that NE axons in the rat PFC exhibit morphological features that deviate from traditional models of monoamine transmission. First, these axons typically lack detectable levels of the rate-limiting enzyme tyrosine hydroxylase (TH); second, the plasma membrane transporter (NET) is primarily localized within the cytoplasm as opposed to the membrane. These features differ markedly from most cortical serotonin or striatal dopamine axons and suggest that NE axons in the naïve rat PFC are not designed for rapid and efficient transmitter synthesis and recycling. It is possible that these features may be fundamentally altered by persistent physiological activation of the LC projection. We tested this hypothesis by examining NE axons in the PFC of rats exposed to chronic uncontrollable stress.
Methods: Rats were subjected to two weeks of continuous cold, a stressor known to persistently enhance electrophysiological activity in LC neurons and to alter neurochemical measures of release in PFC NE axons. Brain tissue from these animals and from control rats was then prepared for dual immunocytochemical detection of NET and TH by electron microscopy.
Results: Chronic cold stress significantly increased the expression of TH and substantially enhanced the translocation of NET to the plasma membrane without affecting the overall levels of NET in NE axons. Moreover, preliminary observations suggest that altered transporter trafficking was specific to NET and was not observed with the serotonin transporter in the same animals.
Conclusion: This study represents the first demonstration of activity-dependent trafficking of NET and TH in the intact CNS. These stress-induced transformations will potently impact monoamine dynamics in the PFC by altering the capacity of NE axons to synthesize and recycle NE. Greater plasmalemmal NET expression will also affect the availability of dopamine, given that dopamine is substantially cleared by NET in the PFC. Whether these structural changes will enhance or degrade the overall functional capacity of the PFC is an important consideration in light of evidence that either reduced or excessive levels of NE and dopamine can degrade cognitive function. Our results have important implications for understanding NE modulation of the cognitive and affective functions of the PFC, the dynamic alterations produced by behaviorally-relevant stimuli, the pathophysiology of stress and mental disorders exacerbated by stress, and the therapeutic efficacy of selective NE reuptake inhibitors (SNRIs) used to treat depression. This work was supported by an NIH grant MH50314 (SRS) and a NARSAD Young Investigator Award (LAHM).

Second Messenger Mechanisms Contributing to Stress-Induced Prefrontal Cortical Dysfunction

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Background: Most neuropsychiatric disorders are worsened by stress exposure, and involve significant dysfunction of the prefrontal cortex (PFC). Exposure to even mild, uncontrollable stress markedly impairs the working memory and inhibitory functions of the PFC. PFC neurons exhibit spatially tuned, mnemonic firing during the delay periods of spatial working memory and inhibitory tasks. Understanding the neural mechanisms underlying stress-induced PFC dysfunction at the

cellular level may elucidate the etiology of mental illness, and identify novel strategies for therapeutic manipulations. Biochemical and cognitive studies to date have revealed the important roles of dopamine (DA) and norepinephrine (NE) release in the PFC. These studies have shown that high levels of DA D1 and NE α1 receptor stimulation during stress dramatically impair PFC cognitive function. The intracellular mechanisms mediating this impairment are of great interest.
Methods: Single units were recorded from PFC in monkeys performing an oculomotor delayed response task. Iontophoresis was used to manipulate intracellular signaling pathways. Infusions were also made into rat PFC to examine effects on PFC cognitive performance. Systemically administered drugs were administered to monkeys where appropriate, e.g. the PKC inhibitor, chelerythrine was administered p.o. prior to cognitive testing.
Results: High levels of DA D1 receptor stimulation suppressed neuronal firing and impaired working memory via cAMP signaling. Thus, high dose D1 agonists, a cAMP analog, or PDE4 inhibition all eroded delay-related cell firing. Firing was restored by the cAMP inhibitor, Rp-cAMPS. Rp-cAMPS similarly restored cognitive performance in stressed animals. High levels of NE α1-adrenoceptor stimulation suppressed cell firing and impaired working memory performance in rats and monkeys. These detrimental effects were reversed by α1-adrenoceptor antagonists such as prazosin, now in use to treat Post-Traumatic Stress Disorder. NE α1-adrenoceptor suppression of delayed-related firing and impairment of spatial working memory was mediated via excessive phosphotidyl inositol (PI) protein kinase C (PKC) signaling. The PKC inhibitor, chelerythrine, restored normal PFC cell firing and protected working memory performance in both rats and monkeys.
Conclusion: Genetic mutations that render cAMP or PKC signaling more potent (e.g. COMT-met-met) may increase vulnerability to PFC deficits in mental illness. The role of PI/PKC signaling in stress-induced PFC dysfunction is of particular interest, as an inhibitor of PI signaling, RGS4, is markedly down-regulated in the PFC of patients with schizophrenia. These data explain why loss of RGS4 may be associated with profound dysfunction of the PFC in schizophrenia, and why agents with PKC inhibitory properties (lithium, valproate, atypical anti-psychotics that block α1 and 5HT2 receptors) have therapeutic effects in several neuropsychiatric disorders.

The Alpha-1 Adrenoreceptor Antagonist Prazosin Improves Combat Veteran and Civilian Posttraumatic Stress Disorder

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Background: Stimulation of prefrontal cortex alpha-1 adrenoreceptors (alpha-1 AR) produces cognitive changes similar to those of PTSD. Together with alpha-1 AR mediated effects on corticotrophin releasing factor secretion and on sleep physiology, these neurobiologic phenomena provide rationale for pharmacologic antagonism of CNS alpha-1 AR as a treatment approach for PTSD. Prazosin is the only clinically available alpha-1 AR antagonist that is lipid soluble and has been demonstrated to block CNS alpha-1 AR after peripheral administration. Clinical trials were performed to evaluate prazosin efficacy for difficult to treat PTSD trauma nightmares and sleep disruption and for daytime symptoms.
Methods: Ten Vietnam combat veterans participated in a placebo-controlled 20-week prazosin augmentation crossover trial (mean dose = 9.5 mg at bedtime). A follow-up 8-week placebo controlled parallel group prazosin augmentation trial was performed in 40 Vietnam combat veterans (mean dose = 13.5 mg/bedtime). We also determined if additional daytime prazosin would reduce "residual" daytime PTSD symptoms in persons with civilian trauma PTSD whose trauma nightmares and sleep disturbance had been reduced with bedtime prazosin (the duration of prazosin action is only 4-10 hours). Prazosin or placebo were administered in early afternoon in a double-blind crossover design to 11 subjects (8 women, 3 men) and effects on response to the Emotional Stroop paradigm determined.
Results: Prazosin was significantly and substantially superior to placebo for primary outcome measures in all studies. Reduction of Clinician Administered PTSD Scale (CAPS) "nightmare" item and "disturbed sleep" item scores and improvement in Clinical Global Impression of Change (CGIC) all favored prazosin with effect sizes of 1.4 or greater. Total CAPS score improvement also favored prazosin (effect size 0.7). In the larger parallel group study prazosin produced significant and large effect size improvements favoring prazosin (mean dose = 13.5 mg at bedtime) on CAPS nightmare scores, Pittsburgh Sleep Quality Index and CGIC. Total CAPS score numerically favored prazosin. In addition, prazosin changed dream quality toward "normal" dreaming and there was a trend favoring prazosin on the Hamilton Depression Rating Scale even after sleep-related depression items were removed. Reductions in POMS "emotional distress" item score following E-Stroop significantly favored prazosin. Preliminary data using "nightcap" home sleep monitoring demonstrate increased sleep duration with prazosin. Prazosin was well tolerated in all studies.
Conclusion: Bedtime prazosin is effective for PTSD trauma nightmares, sleep disruption and global clinical status regardless of trauma etiology. Because of its short duration of action, prazosin often must be administered twice or three times daily to reduce daytime reexperiencing and hyperarousal PTSD symptoms in addition to trauma nightmares and sleep disturbance. CNS alpha-1 AR antagonism with prazosin is a useful and inexpensive approach to PTSD treatment. These results also suggest that chronically increased CNS alpha-1 AR responsiveness contributes to PTSD pathophysiology.

Panel Session
Molecules, Methods and Memory: Research Update on Alzheimer's Disease Therapeutics from the ADCS

Effects of Docosahexaenoic Acid (DHA) in Slowing the Progression of Alzheimer's Disease

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Abundant evidence from epidemiologic and animal studies support the use of DHA, an omega 3 fatty acid as a treatment for Alzheimer Disease (AD). Reduced risk of AD has been associated with DHA consumption and studies of murine models of AD show that dietary DHA reduces brain levels of beta amyloid (A beta mediated oxidative damage and neurotoxicity. We propose a multi-center double blind placebo controlled parallel arm clinical trial of DHA to slow the progression of AD. Participants will be limited to subjects consuming less than 200mg DHA per day which is 300% of that in the average American diet. A dose of 2gms /day is proposed based on evidence that plasma levels of DHA are maximized in most subjects by this dose and based on evidence that this does is safe. 400 subjects will be studied with 60% assigned to active ingredient and 40% to placebo to enhance recruitment. Treatment duration will be 18 months. Dual primary endpoints are rate of decline on ADAScog and rate of decline of CDR-SOB. Functional and behavioral outcomes will also be assessed. Compliance with study drug, other relevant changes in diet and hypothesized therapeutic mechanisms will be assessed with a panel of biomarkers. Serial plasma levels of DHA will be used to measure compliance, with supplementation variability in absorption and possible changes in dietary intake of DHA during the study. Based on animal studies, the mechanisms of action of DHA include anti-amyloid, anti-oxidant and neuroprotectant effects. Volumetric brain MRI determinations of brain atrophy rate in a subset of subjects will be used to detect neuroprotectant effects. Cerebrospinal fluid levels of beta amyloid and lipid peroxidation products in a subset of subjects will be used to detect anti-amyloid and antioxidant effects. Plasma and urine markers of oxidative damage and plasma lev-

els of AD relevant micronutrients will also be obtained in order to monitor other changes in dietary habits which can confound detection of DHA effects. Based on a conservative rate of decline in the placebo group (3.8points/yr on the ADAScog) this study will have 90%power to detect a 38% or larger reduction in the rate of ADAS-cog decline between DHA and placebo and 80% power to detect a 33% effect.

Intravenous Immunoglobulin (IVIg) for the Treatment of Alzheimer Disease

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Antibodies against the amyloid beta protein have demonstrated a remarkable ability to arrest and even reverse key elements of the neuropathology of Alzheimer 's disease (AD). Unfortunately attempts to treat AD by active vaccination failed to generate anti A-beta anti-bodies in a majority of the patients and caused severe brain inflammation in others. Passive immunization (administration of antibodies from an exogenous source) can more consistently deliver therapeutic antibodies without many of the safety and tolerability issues associated with active vaccination. Intravenous immunoglobulin (IVIg) is a very promising agent for this purpose. IVIg is a purified immunoglobulin preparation with unique immune modulating properties that was found to contain elevated titers of polyclonal A-beta antibodies. IVIg's established safety record from over 25years of clinical use as an FDA approved treatment for immune deficiency and autoimmune disorders will reduce the time and risks associated with testing in AD. In 3 pilot studies of mild to moderate stage AD, IVIg treatement for 6 to 12 months significantly improved dementia symptoms and was generally well tolerated. In addition, IVIg increased plasma A-beta antibody titers and promoted clearance of A-beta from the spinal fluid. Ongoing phase II studies will provide further information about IVIg's pharmacokinetic profile and mechanisms of action. A large scale controlled prospective clinical trial is needed to determine if IVIg is useful for treating AD. We will carry out a 30 week double blind placebo controlled randomized 30-center clinical trial examining whether IVIg is effective and well tolerated for treating mild to moderate AD. 210 AD patients will be randomized to receive intravenous infusions of either IVIg (0.4g/kg) or a physiologic saline solution every 2 weeks for 6 months. Outcomes will be measured at baseline, after 12 and 24 weeks and 6 weeks after the final infusion (i.e. week 30). The primary outcome will be the mean change from baseline to 24 weeks on the ADAS-cog and the ADCS Clinical Global Impression of Change (ADCS-CGIC). Levels of A-beta protein and anti-A-beta antibody titers in plasma will be measured at 4 time periods to test the possible correlations with IVIg treatment. Other secondary outcome measures will include the Neuropsychiatric Inventory as a measure of behavioral change, the ADCS Activities of Daily Living Scale (ADCS-ADL) to assess functional status and he ADCS pharmaco-economic Inventory as an index of cost effectiveness. Compliance and adverse events will be carefully monitored through out the trial. This study has been powered to provide 80-90% likelihood of detecting significant differences in the primary outcome measures after 6 months of double blind treatment.

A Randomized, Double-Blind, Placebo-Controlled Trial to Assess Biomarker Outcomes, Safety, and Tolerability of Lithium Carbonate in Outpatients with Probable Alzheimer's Disease

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Lithium (Li) has been considered as a novel treatment for Alzheimer's disease (AD). Li's intriguing biological activities, especially upregulation of Bcl-2, inhibition of GSK-3 and regulation of A-beta, hold promise for long-term therapeutic benefit in AD. This approach is topical now given the emerging data about the potential relevance of apoptosis, altered neurogenesis, and abnormal protein phosphorylation in AD. However, we lack adequate evidence of a biological signal indicative of a therapeutic effect in patients with AD. Concerns regarding toxicity, dosing, and side effects have also limited its study. We therefore propose a pilot study in patients with AD that is sufficient in scope to assess the impact of Li on biomarkers selected for their relevance to the mechanism of action of Li and the pathobiology of AD. In addition, the pilot study will help clarify tolerability, safety, and feasibility issues relevant for consideration of a larger subsequent trial. The pilot study may be used as a stepping-stone to a larger and longer multicenter trial in patients with AD or mild cognitive impairment. 70 patients with mild-moderate probable AD will be randomized to treatment with Li at doses sufficient to achieve a target level of 0.4-0.8 meq/L or placebo in a double-blind manner for 12 weeks. Lumbar punctures will be performed at baseline and after 12 weeks of blinded therapy. A visit will occur 4 weeks after cessation of therapy to assess for possible untoward effects of Li therapy or lumbar puncture. Our primary hypothesis is that Li treatment will produce detectable and reproducible alterations in CSF total tau and p-tau-181 levels in comparison to placebo treatment. The sample size was chosen to achieve a sample of 60 study completers; we will have 90% power to detect a 24.1 % decrease in mean CSF t-tau over 12 weeks (80% power to detect a 20.8% decrease), and 90% power to detect a 24.9% decrease in CSF p-tau181 (80% power to detect a 21.4% decrease). Secondarily, we also hypothesize that Li will produce detectable and reproducible changes in CSF A-beta 1-40 and 1-42 and that Li will be safe and well-tolerated at low doses. We will explore effects of Li on plasma A-beta and on other CSF measures of relevance, including isoprostanes.

A Pilot Phase 2 Study to Evaluate the Impact on Biomarkers of Resveratrol Treatment in Patients with Mild to Moderate AD

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A phase II-a study will evaluate the impact of resveratrol treatment on clinical outcomes and on putative biomarkers in patients with mild-to-moderate dementia due to AD. Patients will receive either resveratrol (1 g po twice daily) or identical placebo in addition to stable F.D.A.-approved medical management. The study design will be a double-blind, placebo-controlled trial conducted at ADCS clinical centers. A total of 90 patients (45 per arm) with mild to moderate AD will be randomly assigned to treatment with resveratrol or matching placebo for a duration of 12 months. A subset of 60 subjects will undergo lumbar puncture to probe putative CSF biomarkers. Randomization will be stratified by site. Cognitive parameters will be assessed to probe symptomatic improvement in: Alzheimer's Disease Assessment Scale (ADAS-cog), MMSE, ADCS-Clinical Global Impression of Change (ADCS-CGIC), ADCS-ADL, Clinical Dementia Rating Scale-Sum of Boxes (CDR-SOB), and Neuropsychiatric Inventory (NPI). Blood samples from patients will be analyzed for ApoE genotype. Serum resveratrol levels will be assessed at baseline and at visits 3, 4, 5, and 6. Biomarkers of AD will be measured in CSF before and after 12 months administration of resveratrol. Plasma and CSF samples will also be banked for analysis of putative AD biomarkers currently in development stages. The Primary aims are: 1. To assess the effect of a fixed dose treatment of resveratrol on putative biomarkers of Alzheimer's disease (AD) (particularly CSF total tau, but also CSF Abeta42, CSF Abeta40, and CSF phospho-tau181), and 2. To assess the safety and tolerability of treatment with resveratrol over a 12 month period in subjects with mild to moderate AD as assessed by analysis of adverse events, including symptoms, abnormal findings on physical examinations, and standard laboratory tests. The Secondary aims are: 3. To explore the effect of treatment with resveratrol on MMSE, ADAScog, ADCS-CGIC, ADCS-ADL,

CDR-SOB, and NPI in subjects with mild to moderate AD, and 4. To examine the influence of ApoE genotype on the effects of resveratrol treatment.

Panel Session
New Bioinformatics Approaches for Neuropsychopharmacology

Bio-ontologies for Neuroscience

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Advances in neurophysiology and neuropharmacology depend upon the ability of biomedical researchers to integrate and interrogate large amounts of heterogeneous genomic data. Bio-ontologies support standard data descriptions for genetic, genomic and phenotypic data, facilitate data integration and exchange among bioinformatics and resources providers, and enhance the ability of scientists to analyze large data sets and to utilize comparative genomic and phenotypic information in their research. One significant advantage of applying bio-ontologies to genome scale data is that biological knowledge can be represented in formats that are human-readable and machine-computable. Our work focuses on the development and implementation of bio-ontologies such as the Gene Ontology, and the use of GO and other bio-ontologies in the Mouse Genome Informatics (MGI) database. MGI is the community model organism database for the laboratory mouse. MGI seeks to support the use of the mouse as a model system for understanding human biology through the integration of genetic and genomic data. We will describe the use of bio-ontologies for annotation, integration, and visualization of biological information for the laboratory mouse. We will focus on the development and use of the Gene Ontology and recent extensions of GO to facilitate the representation and recovery of information about neurological systems.

Rapid Compilation of Neurobiological Networks from Base-Pair to Behavior

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Discovery of gene to phenotype networks for neuropsychopharmacology is an exciting new approach to the identification of novel biological mechanisms and candidate therapeutic targets for behavioral disorders. Recent advances in biocomputational science, high-throughput molecular phenotyping, systems neurobiology and mouse genetics are now readily incorporated into an integrative approach for neurobehavioral investigation which we refer to as "systems genetics". The conceptual framework is simple. Members of a reference population are characterized on diverse traits including SNP alleles, mRNA abundance in multiple tissues and conditions, and biobehavioral traits across all levels of scale. The data are then integrated through linkage analysis, genetic correlation analysis and combinatorial network analyses. We have assembled a system of databases and analytic tools that allow users to construct and analyze the biological networks around any gene or phenotype of interest (www.genenetwork.org). Examples include the dopamine receptor Drd4 abundance or cocaine induced exploratory activity. The location of regulatory polymorphisms and the genes they affect can be detected, the molecular networks around the trait can be defined, and the set of related phenotypes can be identified. Graph theoretical approaches allow extraction of networks from the entire set of genes, polymorphisms and phenotypes, and allow integration of relations among neurobiological and behavioral traits.

Mouse Neuronal Expression Database

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In order to understand the diverse signaling pathways that mediate drug effects within distinct neural cell types, it is critical to know precisely which proteins are coexpressed within these cell types. For example, two different cell-types may express the same receptor, but may have very different responses to its activation by virtue of expressing a different set of downstream signaling molecules and effectors. This type of "coexpression" information is difficult to obtain by gene expression profiling of tissue homogenates, which does not maintain cell-type specificity, or by in situ hybridization or other "gene-by-gene" methods. We have recently developed methods for obtaining global expression profiles of specific neuronal cell-types using Affymetrix gene chips. Our approach utilizes hand-sorting of fluorescently labeled neurons obtained either following tracer injections into projection targets, or from transgenic mouse lines in which specific neuronal cell types express fluorescent proteins. The populations profiled include several sub-types of glutamatergic pyramidal neurons, GABAergic interneurons and catecholaminergic neurons in cortex, thalamus, hippocampus, amygdala, substantia nigra and locus coeruleus. We have made this dataset available via a website (http://mouse.bio.brandeis.edu). We have also used the data to develop a new scheme for classifiying neuronal cell types based on genome-wide expression profiles. The majority of genes associated with cell-cell signaling and cell-cell interactions exhibit cell-type specific expression, and many of these are specifically expressed in restricted populations. Promoters of those genes should be useful for manipulating specific subpopulation of neurons. The neuronal expression database can be mined for predictions about the specific signaling pathways operational in each of the profiled cell types.

Using Whole Tissue Microarrays and Cell Type - Specific Gene Expression Databases to Define Cellular Identity of Brain Plasticity

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A wealth of data on wide-scale gene expression in brain associated with various genetic, pharmacological and pathological perturbations is becoming available for data mining. The emergence of novel bioinformatics resources encourages re-evaluation of existing data sets and allows researchers a different perspective. For example, gene expression databases that characterize transcriptional fingerprints of individual cell types recently became available. We combined the sensitivity of whole tissue microarrays with the specificity of single cell transcriptomes to define transcriptional patterns of brain functions with cellular resolution. We used cDNA microarrays to identify transcriptional fingerprints of cellular plasticity in response to a genetic deletion of the $\alpha 1$ subunit of GABAA receptors. First, transcripts differentially regulated between $\alpha 1$ knock-outs and wild type controls were identified in cerebral cortex and cerebellum. We then used public gene expression databases to identify subsets of transcripts enriched in excitatory and inhibitory neurons as well as some glial cells, which provided evidence for cellular plasticity in individual cell types. In particular, we used the Mouse Neuronal Expression Database (http://mouse.bio.brandeis.edu, presentation by Dr. Sugino) to classify mutation-driven transcriptional changes into seven neuronal categories in cerebral cortex. We also used the Allen Brain Atlas (ABA, http://www.brainatlas.org) to determine cellular identity of regulated transcripts in cerebellum of GABAA $\alpha 1$ null mutants. Gene Ontology (GO) Consortium (http://www.geneontology.org, presentation by Dr. Bult), BioCarta pathways (http://www.biocarta.com/genes/index.asp), and Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg) were used

to classify cell type specific transcripts into functional categories. The GeneNetwork databases (www.genenetwork.org, presentation by Dr. Chesler) were also used to calculate genetic correlations between regulated transcripts and behavioral phenotypes observed in the null mutants. Further analysis linked some transcriptional changes to cellular phenotypes observed in the knock-out mice and suggested several genes, such as the early growth response 1 (Egr1), small GTP binding protein Rac1 (Rac1), neurogranin (Nrgn), sodium channel β4 subunit (Scn4b) and potassium voltage-gated Kv4.2 channel (Kcnd2), as cell-type specific markers of neuronal plasticity. Furthermore, transcriptional activation of genes enriched in Bergman glia suggests an active role of these astrocytes in synaptic plasticity. Overall, our results highlight the importance of convergent neuroinformatics approaches to study transcriptional regulation of complex traits. Supported by grants from National Institute of Alcohol Abuse and Alcoholism, NIH (AA UO1 13520, AA UO1 13518, AAUO1 13475; INIA Projects).

Panel Session
Time Course of Drug and Placebo Response: Implications for Clinical Trials and Drug Discovery

Early Improvement as a Predictor of Treatment Outcome with Antidepressants: Why the First 2 Weeks Really Matter

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Background: Clinicians currently assume that most antidepressants have a delayed onset of efficacy. Therefore early clinical improvement that may occur during the first 2 weeks of treatment is usually not regarded as important for the assessment of treatment outcome. However, the delayed onset hypothesis has been questioned by recent data.
Methods: We analyzed the time course of improvement and response of individual patients with Major Depression treated in double or single blind controlled trials with different antidepressants or placebo (Organon data base), as well as the utility of early improvement during individual treatment course as a predictor of later treatment outcome. Improvement was defined as a HAM-D-17 (ITT group: n=6545) or MADRS (ITT-group: n=3828) score reduction of >= 20%. Stable response (≥ 50% reduction in HAM-D from baseline) or remission (HAM-D total score reduction to ≤ 7) was defined as being present both at week 4 and week 6.
Results: Our results yielded clear evidence that if improvement occurred, it occurred in a majority of patients within the first 2 weeks of treatment, particularly in later stable responders/remitters, and that it predicts later stable response or remission with high sensitivity. In patients not showing improvement by at least 20% after 2 weeks of treatment, the clinician would have been wrong only in approx. 10% of patients (false negative rates for stable response remission), if relying on early improvement as the predictor to adjust treatment in this early stage of antidepressant treatment. Comparable results have been found for the trials using the MADRS scale. Moreover, this 20% early improvement parameter showed excellent sensitivity as a predictor of stable response or remission in a prospective trial comparing mirtazapine with venlafaxine in major depression examining onset of action.
Conclusions: These empirically derived data suggest that the early individual course of improvement is of major relevance for a patient's individual treatment outcome and provide important clinical clues for an individually tailored antidepressant treatment. The results indicate that early improvement is a very common phenomenon in patients subsequently responding to treatment (though early improvement is no guarantee for later response or remission). For patients not reacting to treatment after 2 weeks there is an important clinical implication: if a patient has not shown an improvement after 2 weeks, there is little chance that she/he will nevertheless become a responder or remitter with unchanged treatment within 6 weeks, and a change of treatment strategy at this stage seems justified in order to reduce unnecessary prolongation of patients' suffering.

The Time Course of Antipsychotics Versus Placebo: On Overall Efficacy, Positive, Negative and Cognitive Symptoms

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Background: This paper will characterize antipsychotic-induced improvement in schizophrenia in comparison to placebo by analysis of a number of large random controlled trials (RCT). We will also explore whether the time course of drug versus improvement of different types of symptoms, positive symptoms, negative symptoms, cognitive symptoms, affective symptoms, etc. have the same or different time courses. Also examined will be the degree to which prediction of drug versus placebo final improvement score at the end of the trial can be made from initial improvement at 3 days, 1 week, 2 weeks, etc. Although some improvement may occur later in the trial, we evaluate whether it is entirely predictable from earlier improvement, and consequently carries little information in distinguishing drug from placebo.
Methods: Multiple double-blind placebo-controlled RCT will be examined to determine the relative rate of improvement of drug versus placebo. These include the NIMH collaborative study number 1, the first large collaborative study to be done by Jonathan Cole and his coworkers at NIMH in the early 1960s on acutely ill patients, many of whom were first episode. We will also use the registrational studies of risperidone done in the United States and Canada, and the registrational studies of olanzapine. Johnsone, Owens, and Crow performed a random-assignment double-blind study of 120 patients, where patients were measured every 3 or 4 days. This provides a more detailed assessment of rate of improvement than is provided by studies which only assist patients every week. We will also do a meta-analysis of loading dose/acute administration studies where patients are studied with high initial doses to determine whether an initial high dose can shorten the time of response. We will also compare symptomatic improvement using a direct measure of thought disorder in schizophrenia. We will compare the rate of improvement produced by second- and first-generation antipsychotics.
Results: Most patients with schizophrenia show benefits relatively early even in the first few days and certainly by one week. Many show almost, most or even all the benefit that they will eventually achieve by two weeks.
Conclusions: The time course of antipsychotic action in comparison to placebo is presented and is found to be much briefer than is implicitly implied in the present thinking that there is a delay period in trials of 6-8 weeks are necessary. It is clearly feasible to assist the acute action of antipsychotics with trials as short as two weeks. The overall aim will be to explore how a detailed exploration of drug placebo difference over time can identify the length of trial necessary to prove efficacy. This could result in shorter more cost effective trials while lessening the exposure to placebo. This will complement an analysis of Carpenter who will focus on methods to reduce the exposure to placebo while maintaining statistical power. This also has implications toward mechanism of action and consequently drug discovery.

The Early Onset of Antipsychotic Action – Understanding the Mechanism

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Background: While it is well accepted that all antipsychotics block the dopamine D2 receptor, how this blockade of D2 receptor-mediated transmission leads to the resolution of psychosis is far from clear. Traditionally, it has been thought that there is a prolonged gap between the start of treatment and onset of improvement – the data presented at this symposium show that this is not the case. This

reconceptualization of clinical improvement has important implications for understanding antipsychotic mechanism. We report the results of parallel lines of clinical and preclinical investigation that have examined the mechanisms of this early antipsychotic improvement.
Methods: In a double-blind study, patients with schizophrenia were randomized to treatment with varying doses of risperidone (0.5/3 mg/d) or olanzapine (5/15 mg/d). After two weeks of treatment, their striatal (11C-Raclopride), frontal, thalamic and temporal (11C-FLB457) D2 occupancy was measured and correlated to clinical response as measured using PANSS.
Results: There was dose-dependent range of occupancies from 4 to 95%, the occupancies across different regions were modestly correlated (r = 0.7-0.8). The striatal D2 occupancy was the most robust predictor of early antipsychotic response (r = 0.63, p = 0.1); although it did not significantly predict negative symptom response. The extrastriatal D2 occupancy did not predict antipsychotic response.
Background: It has previously been shown that all antipsychotics show efficacy in the Conditioned Avodance Response model, however, most of these studies have used single doses of antipsychotics. To address the issue of onset and progressive accumulation of antipsychotic effect we decided to study the effect of repeated daily antipsychotic treatment on the CAR model.
Methods: A shuttle-box automated CAR apparatus was used to examine the effects of repeated treatment with Haloperidol, Risperidone, Olanzapine and Chlordiazepoxide in Male SD rats. To understand that underlying behavioural processes – studies were undertaken which manipulated drug exposure, testing intervals, testing conditions, and drug withdrawal.
Results: We found that antipsychotics are effective in CAR at doses that lead to clinically relevant occupancies (~ 60-70%). As in the clinic, the antipsychotic effect in CAR starts after the first dose, grows with time and then asymptotes. This effect is seen with typical (haloperidol) and atypical (risperidone, olanzapine) antipsychotic dosing and is not seen with repeated sedative dosing (e.g. chlordiazepoxide). This progressive increase of the effect of antipsychotics in CAR was not a function of dose accumulation or motor dysfunction, but, reflected a drug-mediated, experience-dependent ability to ignore an aversively conditioned stimulus.
Discussion: The clinical PET data show that antipsychotics lead to high levels of D2 occupancy in striatal and extrastriatal regions within the first few days. This occupancy is followed by an early onset of response, which is correlated with blockade of the striatal D2 receptors. A similar onset and trajectory can also be modeled in the CAR model in animals – where it seems it is the ability of the antipsychotics to reduce the salience of the aversive conditioned stimulus that gives rise to their efficacy. Obviously, CAR is not psychosis, however, the data taken together lead to a plausible suggestion that the antipsychotics, via their blockade of the dopamine system, may reduce the salience of psychotic stimuli in patients – thus leading to an early improvement in the symptoms of psychosis.

New Designs for Placebo Controlled Studies

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Background: To document the effectiveness of a new approach to placebo-controls in RCTs. The intent is to facilitate the use of placebo-control designs by increasing experiment wide safety and decreasing non-random attrition. We will determine the effectiveness of two approaches for testing efficacy hypotheses for antipsychotic drugs. First, how does reduction in trial length effect power to detect drug/placebo difference and estimate of effect size. Second, we will determine the effect of progressive, random reduction in the number of placebo subjects on estimate of placebo effect and power to detect the drug/placebo difference. We hypothesize that not all subjects assigned to placebo are required for the full length of the trial. Rather, all subjects might inform the initial two week data point with progressive and random removal thereafter [in contrast to the non-random attrition common in trials].
Method: In a preliminary analysis we determined that drug/placebo differences were detected in multiple runs dropping 20% of placebo subjects at random at each data point. The next step was to access data from two six-week antipsychotic drug studies with placebo-controlled designs (N=108 placebo; 172 olanzapine). We estimated the percent of the full treatment effect [drug and placebo] observed at each week during the combined studies. We then determined the effect on power to detect an efficacy signal at week two and week four. A planned analysis will determine the optimal approach to reducing the length of placebo exposure in randomly selected subjects [i.e., cost in power or erroneous estimation of observed placebo effect]. The derived approach will be tested in an independent data set.
Results: In the combined data, much treatment effect was observed early with approximately 70% of the final treatment effect occurring by the second week for drug, but also for placebo. Doubling the sample size would be needed to run a two week trial with 80% power to detect a difference with an effect size between 0.5 and 1. At week 4, 86% of the full treatment effect was noted which corresponds to a 36% increase in sample size to detect a difference with the same power. Alternatively, increasing the randomized sample by 15% and decreasing the placebo exposures by 25% would also provide similar power to detect a difference at six weeks. Planned analyses will determine the feasibility of random assignment of placebo subjects to different lengths of placebo exposure. This will address the second issue of maintaining full length of a clinical trial, but reducing experiment-wide placebo exposure.
Conclusions: Criticism of the ethics of placebo-controlled studies has reduced feasibility. When conducted, high rates of non-random attrition reduce the value of the placebo-controlled design. LOCF analyses inflate the placebo effect estimate. Shortening study periods and/or decreasing the number assigned to placebo reduces placebo exposure with definable effect on power. Also, random assignment of placebo subjects to different lengths of study participation could reduce attrition and enhance experiment-wide safety.

Panel Session
Translating Research on the Metabolic Effects of Antipsychotics into Public Health and Treatment Guidelines

Effects of Atypical Antipsychotics on Adiposity and Pancreatic Function Suggest Mechanism for Drug-Induced Diabetes

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Background: The reported link between atypical antipsychotic use and development of diabetes has focused attention on the pathogenesis of treatment-emergent metabolic dysfunction. Weight gain is a common side effect of antipsychotics, and often results in reduced ability of insulin to normalize circulating glucose levels ("insulin resistance"), and it has been suggested that increased body weight and attendant resistance are causal for development of diabetes during drug treatment. However, drug-induced insulin resistance alone is unlikely to cause diabetes because of the known closed-loop relationship between insulin-sensitive tissues (e.g. muscle and adipose tissue) and the insulin-secreting β-cells of the pancreas. Insulin resistance normally elicits a robust compensatory upregulation by the β-cells to maintain glucose tolerance. Thus, diabetes is believed to require the development of multiple defects — insulin resistance and inadequate β-cell compensation – for overt disease to emerge.
Methods: To test effects of antipsychotics on this constellation of metabolic factors (insulin resistance, pancreatic function), plus body weight and adiposity, we performed placebo-controlled studies of olanzapine (OLZ) and risperidone (RIS) in dogs treated for 6 weeks. At baseline and after treatment, we measured total trunk and region-

specific (visceral and subcutaneous) adiposity by abdominal magnetic resonance imaging. Prospective changes in insulin sensitivity and pancreatic β-cell function were assessed by classic and highly quantitative methodologies.
Results: OLZ caused weight gain (+5.9±1.2%, p=0.001) which was not different from placebo (+4.8±1.0%. p=0.5 vs OLZ). There was no significant increase in weight with RIS (+3.9±2.1%, p=0.098). Yet body weight per se did not reveal striking differences between agents upon fat deposition. Changes in adiposity with RIS were not different from that observed in placebo group (p>0.33). In contrast, OLZ resulted in profound increase in adiposity: total body fat was nearly doubled (+91±20%; p=0.000001), reflecting marked increases in subcutaneous (+106±24%; p=0.0001) and visceral (+84±22%; p=0.000001) adipose stores. OLZ increases in adiposity greatly exceeded those observed after RIS in total (18.5±1.8 vs 9.9±2.7 cm^3; p=0.018) and subcutaneous (9.8±1.5 vs 4.0±1.8 cm^3; p=0.024) depots. OLZ-induced obesity was accompanied by severe (↓75%) decrement in insulin's ability to suppress endogenous glucose production ("hepatic insulin resistance"; p<0.009 vs baseline) not evident in RIS- or placebo-treated dogs (p>0.1). In the face of such insulin resistance, pancreatic β-cell function should be enhanced, and this was evident in a separate groups of dogs in which comparable insulin resistance and obesity was induced by high fat feeding rather than antipsychotic treatment (baseline: 0.7±0.2, post-fat feeding: 2.2±0.6 μU/ml per mg/dl; p=0.01). However, OLZ-treated animals failed to demonstrate β-cell upregulation (pre: 1.2±0.2, post: 1.1±0.3 μU/ml per mg/dl; p=0.6), indicating that this antipsychotic interfered with the pancreatic insulin secretory response necessary to compensate for drug-induced insulin resistance and obesity. Drug-induced metabolic abnormalities were independent of observed changes in body weight.
Conclusions: Atypical antipsychotics induce differential metabolic sequelae in the absence of underlying psychiatric disease or comorbidity. These data provide important insights into the mechanisms by which antipsychotics may cause diabetes in the psychiatric population. Further studies are required to determine the mechanisms by which observed defects may develop, and to quantify the effects of other atypical antipsychotics on adiposity and pancreatic function.

Measuring Medication Effects on Regional Adiposity and Tissue-Specific Changes in Insulin Sensitivity

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Background: Growing evidence suggests that antipsychotic medications can impact insulin sensitivity. Increased adiposity can disturb glucose and lipid metabolism via disturbances in insulin sensitivity, and schizophrenia patients experience an increased prevalence of diabetes mellitus and the metabolic syndrome in comparison to the general population. Increased adiposity, plasma glucose and lipids are risk factors for cardiovascular disease (CV), and schizophrenia patients experience higher rates of CV.
Methods: Adiposity can be directly measured using whole body dual energy x-ray absorptiometry (DEXA) and abdominal magnetic resonance (MRI) imaging. These adiposity measures can be analyzed in relation to whole-body glucose and lipid kinetics measured with stable isotope tracer methodology during hyperinsulinemic-euglycemic clamp conditions. These sensitive methods can be used to measure tissue-specific changes in insulin sensitivity that might occur independent or dependent on fat mass. In an ongoing NIMH-funded study, schizophrenia patients undergo prospective randomized assignment to 12 weeks of treatment with olanzapine, quetiapine, risperidone, or ziprasidone, with no other medication changes allowed. In a related NIMH study, subjects undergo 12 weeks of placebo-controlled divalproex augmentation of antipsychotic treatment with measurement of adiposity and insulin sensitivity measures.
Results: Interim analysis of the antipsychotic monotherapy study indicates treatment effects on adiposity as well as predicted relationships between adiposity and tissue-specific insulin sensitivity. A significant time X treatment condition (olanzapine, quetiapine, risperidone, or ziprasidone) interaction was detected for DEXA total fat (F[3,15]=9.48, p=0.0009), MRI total abdominal fat (subcutaneous plus visceral fat) (F[3,8]=5.88, p=0.02), and MRI subcutaneous fat (F[3,8]=4.90, p=0.032), with a trend level interaction for MRI visceral fat (F[3,9]=3.21, p=0.076). No significant time X treatment group interaction was detected for DEXA lean body mass (F[3,15]=1.70, p=0.21). Glucose Rd (rate of disappearance of glucose into muscle) was predicted by DEXA total body fat (F[1,15]=13.49, p=0.0023). Glycerol Ra (rate of lipolysis in fat) was predicted by MRI visceral fat (F[1,13]=15.67, p=0.0016). Glucose Ra (rate of hepatic gluconeogenesis) was predicted by DEXA total body fat (F[1,15]=4.44, p=0.05). Covarying baseline DEXA total fat mass (to control for baseline differences in fat), significant time X treatment group interactions for glycerol rate of appearance (Ra) (F[1,3]=194, p=0.005) were observed. These results support the hypothesis that increases in fat mass are generally associated with decreases in insulin sensitivity. These effects are likely generalizable to other psychotropic treatment conditions associated with weight gain, as evidenced by the divalproex augmentation study. Preliminary results of this study indicate significant increases in weight associated with divalproex augmentation expressed as increases in abdominal fat measured by MRI (F[1,18]=13.56, p=.002). Associated with this increase in abdominal fat, increases in fasting total cholesterol (p=.006), LDL (p=.039), and triglycerides (p=.03) were observed.
Conclusion: Sensitive gold-standard measurements can be used to evaluate medication-induced changes in body composition, and glucose and lipid metabolism. Increases in adiposity are associated with predictable reductions in insulin sensitivity, which are relevant to the risk of diabetes and cardiovascular disease. Support Contributed By: NARSAD (Stephen and Connie Lieber Young Investigator Award), NIMH K23 MH 067795; NIH R01 63985, USPHS, #MOIRR00036, GCRC, CNRU P30 DK56341, P60-DK20579 and Abbott Laboratories.

Approach to Screening and Prevention of Type 2 Diabetes

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Background: Type 2 diabetes (T2DM) is frequently not diagnosed until complications appear. The purpose of screening is to identify asymptomatic individuals who are likely to have diabetes. Individuals felt to be at high risk in particular should be screened for T2DM (or pre-diabetes). This is referred to as "targeted screening" and differs from "population screening" where all persons within a given population are screened. Separate diagnostic tests interpreted using published criteria are required after positive screening tests to establish a definitive diagnosis. The 2 hour oral glucose tolerance test identifies people with both impaired fasting glucose and impaired glucose tolerance (IGT), and thus more people at increased risk for the development of diabetes, the fasting plasma test is considered more convenient for patients, more reproducible, less costly, and easier to administer than the glucose tolerance test. An emerging area of research concerns the use of other tests, such as the hemoglobin A1C, both for screening for and diagnosis of diabetes.
Methods: Population studies, intervention trials and field experience in implementing treatment and monitoring guidelines have provided the basis for ADA recommendations for screening and prevention of T2DM.
Results: Epidemiologic data and intervention studies have indicated that certain subgroups are at additional risk for the development of T2DM and require enhanced monitoring. Currently identified high risk characteristics include: habitual physical inactivity, having a first-degree relative with T2DM, being a member of a high risk ethnic population (e.g. African-American, Latino, Native

American, Asian American, Pacific Islander), having delivered a baby weighing > 9 lbs or having been diagnosed with gestational diabetes, hypertension (BP >140/90 mmHg), HDL cholesterol < 35 mg/dl and/or a triglyceride level >250 mg/dl, history of polycystic ovary syndrome (PCOS), IGT or IFG on previous testing, history of vascular disease. Earlier and more frequent screening is recommended for individuals with these risk factors. Recent evidence suggests that certain psychiatric patient groups may be at increased risk for the development of T2DM, suggesting the value of enhanced monitoring.
Discussion: Data indicate that there are risk factors that predict vulnerability to diabetes and its complications, and that it is feasible to implement enhanced monitoring guidelines targeted at individuals with these risk factors. These results are relevant to evidence that psychiatric subgroups may be at increased risk for T2DM. The ADA's experience suggest a general approach to creating guidelines for monitoring and preventing T2DM in individuals with psychiatric disorders.

American Psychiatric Association Workgroup on Antipsychotics and Metabolic Risk
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Background: Individuals with schizophrenia have elevated rates of mortality and medical comorbidity, related to increased rates of conditions such as type 2 diabetes mellitus and cardiovascular disease. While it is likely that lifestyle issues (e.g., reduced activity, poor nutrition) play a key role, a range of evidence suggests that treatment with antipsychotic medications is associated with an increased risk for insulin resistance, hyperglycemia, and dyslipidemia. An American Diabetes Association (ADA) Consensus Development Conference, co-sponsored by the American Psychiatric Association (APA) and other organizations, recently addressed this topic, and the US FDA recently recommended a package warning concerning hyperglycemia for all second generation antipsychotics, leading to a number of questions about specific conclusions that were reached, the process used, and the implications for treatment decision making.
Methods: The APA Committee on Research on Psychiatric Treatments convened a workgroup to address outstanding questions and to provide additional guidance to the field. Experts in endocrinology, cardiology, psychiatry and services research reviewed relevant literature in their respective areas of interest. Over 60 contributors submitted subsections for review that are incorporated into the report, and over 80 participants will have reviewed and edited the final report. Literature references were identified primarily via Medline searches. The reports identified can be broadly divided into 1) uncontrolled observational studies, 2) large, controlled, observational database analyses using prescription, administrative or – less commonly – population-based databases, and 3) controlled experimental studies, including randomized clinical trials.
Results: On the specific topic of antipsychotics and diabetes or dyslipidemia risk, over 1000 papers are currently in the literature, with a more limited literature of well-controlled experimental studies. The Workgroup will be reviewing the draft report during the month of April, aiming to identify areas of consensus and discrepant results and/or discrepant interpretations that will be incorporated into the final report.
Conclusion: Similar to the ADA's ongoing use of Consensus Development Conferences, the APA Workgroup offers an opportunity to address controversial areas of research with comprehensive expertise in order to identify areas of consensus, discrepant results and directions for future research. The APA workgroup identified opportunities for managing metabolic risk during antipsychotic treatment and improving health outcomes.

Panel Session
An Insular View of Anxiety

The Neuroanatomical Basis for Human Awareness of Interoceptive Feelings from the Body
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Experimental anatomical and physiological evidence will be presented that identifies a phylogenetically unique pathway to the insular cortex of humanoid primates which represents the homeostatic condition of the body. Imaging evidence in humans will be described supporting the view that multiple re-representations of this interoceptive pathway underlie human awareness of self, emotion, music and time. This view is consistent with the expanded version of the James-Lange theory of emotion related in Damasio's "somatic marker" hypothesis of consciousness. These data also fit with recent work on the "Von Economo neurons" that are uniquely present in the fronto-insular and anterior cingulate cortices of humanoid primates. Finally, based on this interoceptive (homeostatic) view of awareness, a new proposal for the forebrain asymmetry of emotion will be presented.

Role of Insula in Emotional Representations: Neuroimaging and Psychophysiological Evidence
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Background: Influential theories highlight an integration of bodily arousal states with appraisal of cognitive context in subjective emotional representations. This model is particularly relevant to the understanding of anxiety states where attention to visceral arousal responses augments subjective feelings of distress.
Methods: Functional neuroimaging combined with psychophysiology and patient studies.
Results: Experimentally, fear-conditioning provides a model of learned anxiety evoking autonomic arousal responses. In a neuroimaging study of a patient lesion-deficit model, regions including amygdala and insula cortex were shown to be sensitive to afferent information relating to visceral arousal, with right insula also reflecting conscious appraisal of threat. In a further study, right anterior insula activity (and gray matter volume) was shown to reflect the quality of interoceptive representations and to predict day-to-day experience of negative affective symptoms, especially anxiety.
Recent studies detail the integrative role of insula cortex in social emotions: First, when perceiving signals of subjective distress, the strength of functional connectivity between right insula and brainstem autonomic nuclei (mediating reactive visceral responses) correlates with empathetic behavioural style. Second, providing subjects with false interoceptive feedback modulates the attribution of emotional intensity to neutral stimuli, and increases neural activity within right insula and adjacent opercular cortex. Finally, activity in these regions is specifically modulated as a function of the degree to which emotions are outwardly expressed.
Discussion: These convergent neuroimaging findings are complemented by evidence from other approaches to suggest a central role of right insula cortex in supporting affective feeling states arising from dynamic integration of salient cognitive representations with visceral and somatic signals.

Insular Hyperactivity in Anxiety Prone Individuals - a New Signature of Anxiety?
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Increased amygdala reactivity during processing of certain types of emotional stimuli has been observed in patients with anxiety disor-

ders such as social phobia and posttraumatic stress disorder. However, the contribution of the insular cortex to anxiety has been less well studied. The insular cortex is a critically component of neural circuits involved in interoception, i.e. the sense of the physiological condition of the entire body. In particular, ascending brain areas include the midbrain reticular nuclei, ventromedial and ventroposterior thalamus, and the interoceptive (posterior) insular cortex, which is integrated in the anterior insular cortex of the dominant (right) hemisphere. We previously found that increased activation in the anterior insular cortex during risk-taking decision-making was correlated to both neuroticism and harm avoidance. The studies that are presented are an extension of these findings to clarify the role of the insular cortex in subjects with high trait anxiety. In the first study, 32 physically healthy subjects 18-21 years old were recruited from a large pool of college students. Of these, 16 were chosen on the basis of scoring in the upper 15th percentile on a measure of trait anxiety (anxiety-prone group), and 16 were chosen on the basis of scoring in the normative range (40-60th percentile). 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. We found that anxiety-prone subjects had significantly greater bilateral amygdala and insula activation to emotional faces than did the anxiety-normative comparison subjects. Higher scores on several measures assessing anxiety-proneness (e.g., neuroticism, trait anxiety, and anxiety sensitivity) were associated with greater activation of amygdala (predominantly left-sided) and anterior insula (bilateral). In the second study, we utilized an anticipation task to examine the reactivity of the insular cortex. Specifically, 16 anxiety prone and 16 anxiety normative individuals performed an stimulus anticipation task in the fMRI scanner during which they viewed pictures of spiders and snakes. Subjects were prompted 4-6 sec before the onset of each aversive image. BOLD signal was measured during the task and functional brain activation during the period when subjects anticipated viewing the images was examined. Anxiety prone subjects showed greater response than anxiety normative subjects in the bilateral insula during anticipation. During the image presentation phase, anxiety normative subjects showed greater activation than anxiety prone subjects in the bilateral temporal lobes and left superior frontal gyrus. Moreover, bilateral temporal lobe activation during image presentation was inversely correlated with bilateral insula activation during anticipation both within groups and in the combined group. These data suggest that greater activation of the insula during visual anticipation is associated with visual processing of aversive stimuli in anxiety prone individuals. In summary, altered insular cortex responsivity to emotional or aversive stimuli appears to play a critical role in mediating increased anxiety.

Corticolimbic Function in Posttraumatic Stress Disorder

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Background: Much of the functional neuroimaging research in anxiety disorders points to increased amygdala activation during emotion processing, accompanied by reduced medial prefrontal cortical (mPFC) activity (presumably reflecting reduced mPFC inhibitory control of the amygdala and, possibly, other limbic structures). Posttraumatic stress disorder (PTSD) is arguably the best-studied of the anxiety disorders from a functional neuroimaging perspective. Anticipation of aversive events (internal or external) and their consequences is believed to drive much of the dysfunction (e.g., phobic avoidance, hyperarousal) in PTSD. We used a task designed to elicit anticipatory anxiety to evaluate the neural substrates of aversive anticipation in women with domestic violence-related PTSD.
Methods: Fifteen women with IPV-related PTSD and 15 non-traumatized control (NTC) women participated in a task in the fMRI scanner during which they viewed emotionally aversive pictures (from the IAPS). Subjects were prompted 4-6 sec before the onset of each aversive image. BOLD signal was measured during the task and functional brain activation during the period when subjects anticipated viewing the images was examined.
Results: PTSD subjects showed greater response than control subjects in the bilateral insula during anticipation. In addition, PTSD subjects had lower activity within the superior/medial frontal gyrus, and demonstrated significant differences in corticolimbic connectivity compared to control subjects.
Conclusions: Enhanced insular activation during anticipation of aversive stimuli is seen in women with domestic violence-related PTSD. These findings will be interpreted and placed in the context of our understanding of the role of the insular cortex in affective processing. Clinical implications will be discussed, and suggestions for future research will be posed.

Panel Session
Causes and Consequences of Inhalant Abuse

Neurodevelopmental Toxicity of Toluene in Rats

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Background: Many women of reproductive age are exposed to solvents like toluene in industrial settings (i.e., relatively constant, low-concentration occupational exposure) or via inhalant abuse (i.e., relatively brief, high-concentration binge exposure). Among commonly abused inhalants, toluene earns particular attention due to its teratogenic and neurotoxic properties. Current research on maternal exposure to inhaled solvents shows that risks for fetal demise or major malformations are much less with low to moderate occupational toluene exposure than with abuse, despite behavioral sequelae for both. There is a greater potential for adverse pregnancy outcomes, developmental delays, and neurobehavioral problems in children born to women exposed to high concentrations of toluene, similar to those encountered in abuse settings, yet the teratogenic effects of binge exposures to toluene and other inhalants remain understudied. Similarly, young organisms (rat or human) may be more sensitive to toluene than older organisms. Animal models aid substantially in clarifying developmental risk of solvent abuse in the absence of other health problems and co-drug abuse, as well as in establishing key dose patterns and critical periods of exposure. Our working hypothesis is that prenatal and pre-adolescent binge exposure to toluene results in negative neural and behavioral sequelae in rats.
Methods: In a clinically relevant animal model of abuse patterns of solvent exposure, timed-pregnant rats were exposed to 8,000 parts per million (ppm) or 12,000 ppm of toluene, or to air (0 ppm), for 15 min twice daily from gestation day 8 (GD8) through GD20. Offspring were tested from postnatal day 4 (PN4) to PN21 in a developmental battery assessing body weight, maturational milestones (i.e., pinnae unfolding, incisor eruption & eye opening), biobehavioral development (e.g., negative geotaxis, surface righting, & grip strength), locomotor activity, and impulsivity. In a separate study using ex vivo high-resolution magic angle spinning 1H magnetic resonance spectroscopy (MRS) at 11.7-Tesla, glutamate (GLU), glutamine (GLN), GABA, and glycine (GLY), as well as NAA, lactate, myo-inositol were quantified on PN21 or PN35 in rats after the same toluene exposure protocol.
Results: Pups exposed in utero to 12,000 ppm toluene weighed significantly less than the control pups at all ages before PN16. There were significant toluene-induced increases in an index of poor perinatal outcome (i.e., a combination of malformations, "runting" and neonatal death), and deficits in negative geotaxis, but no significant delays in reaching maturational milestones. Prenatal toluene

exposure resulted in only small changes in locomotor activity compared to non-exposed rats, but there were significant increases in impulsivity for toluene-exposed rats. Results from the MRS study in hippocampus suggest that at PN21 toluene decreased GABA (both doses) and GLU (only after 12,000 ppm). There were no effects at PN35.
Discussion: The results imply that abuse patterns of exposure are more deleterious than typical occupational patterns of exposure during early development. Abuse of organic solvents during pregnancy in humans may produce long-lasting effects on biobehavioral development. Animal models will continue to be important in studying the expression and mechanisms of developmental organic solvent exposure. Supported by NIDA grants R01–DA015951 and R21–DA019151.

Neurophysiological Mapping of Toluene-Sensitive Cells in the Rat Midbrain
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Background: Inhalation of toluene containing products produces euphoria in humans and functions as a rewarding stimulus in experimental animals. Although the mesolimbic pathway is a common site upon which various drugs of abuse act to produce their rewarding effects, the influence of toluene upon neurotransmission within this pathway remains controversial. One explanation for the existing controversy is that toluene simultaneously generates multiple, confounding effects which vary depending upon the brain concentration, anatomical location, and signaling mediator examined.
Methods: To address this issue and optimize anatomical/ temporal resolution, we employed extracellular single-unit recordings in an in vitro brain slice preparation superfused with behaviorally relevant concentrations of toluene (20 μM-800 μM), and assessed the response of dopamine and non-dopamine (GABA) neurons in the ventral tegmental area, and neurons within the interpeduncular nucleus and retrorubral field and rostral interstitial nucleus.
Results: Data will be presented showing that there are region specific effects of toluene on neuronal activity. Moreover, during extended exposure to toluene the excitatory effects of VTA neurons often progressed to a state of depolarization inactivation which was reversible. Toluene-induced excitations also occurred under conditions where synaptic transmission was inhibited. Neurons within the interpeduncular nucleus, a structure involved in avoidance learning, were uniformly inhibited upon perfusion with toluene. However, retrorubral and rostral interstitial neuronal activity was unaltered in the presence of toluene.
Discussion: Using a technique that favors rapid delivery of known concentrations of toluene to discrete regions of brain tissue, we observed robust and spatially-restricted changes in neuronal firing. That is, toluene activated VTA DA neurons, even under conditions preventing synaptic transmission, and could functionally inactivate inhibitory VTA GABA and IPN nuclei that tonically inhibit mesoaccumbal DA release in vivo. In contrast, toluene was comparatively ineffectual at RRF-DA or RIN neurons. These results reveal that within a narrow concentration range toluene can stimulate mesolimbic neurotransmission by both direct and indirect mechanisms.

Neuroimaging in Animal Models of Inhalant Abuse
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Neurological impairments have been reported following chronic toluene abuse while neuroimaging studies provide evidence of leukoencephalopathy. Inhalants are commonly abused during adolescence. Therefore, we combined PET and MRI in adolescent animals to test the hypotheses that (1) reinforcing doses of inhaled toluene produce reversible changes in FDG uptake and (2) metabolic alterations relate to toluene-induced changes in white matter. PET studies with ^{11}C-toluene identified its distribution. Adolescent male Sprague Dawley rats received FDG and ^{11}C-toluene PET scans followed by MRI scans using a magnetization transfer (MT) contrast (n = 4; microPET R4, CTI; MRI: 9.4T 21-cm horizontal, Magnex Scientific). Animals were conditioned with 5000 ppm of inhaled toluene in a modified conditioned place preference (CPP) chamber. Locomotion was monitored during conditioning. Following 12 exposures, animals received FDG PET and MRI MT scans. Their brains were then stained for myelin using the Weil method. A separate group of animals (n = 8) were scanned to examine recovery of brain function following toluene cessation. Data were spatially preprocessed, normalized to stereotaxic space and segmented. Regional correlations between metabolic change and conditioned behaviors were made using Statistical Parametric Mapping (SPM) t-maps and region of interest (ROI) analyses. PET and MRI images were globally normalized to the mean voxel value and the same ROI template was applied to all scans. Locomotor activity decreased with increasing toluene exposures. ^{11}C-Toluene uptake was highest in pons, colliculi and the internal capsule. Following toluene exposure, MT scans revealed enlarged ventricles in three animals. Whole brain MT ratio (MTR) histogram showed lower peak height and more pixels with low MTR values after toluene exposure. Histological data supported the decline in MTR signal. Regions with highest ^{11}C-toluene uptake showed the marked change in the normalized MTR, whereas the most significant changes in FDG occurred in cortical areas. Whole brain FDG uptake decreased although there was a significant rebound in uptake following toluene cessation. Correlations between the change in MTR and the change in FDG ($R^2 = 0.76$) suggest that a distinct functional versus anatomical profile characterizes the toluene abuse. In conclusion, the regional distribution of [11C]toluene parallels the pathology underlying the functional changes reported following prolonged exposure. At doses significant to produce behavioral tolerance, there are regionally specific changes in brain metabolism that are complimentary yet unique from, those observed in white matter.

Toluene Leukoencephalopathy
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Background: Solvent inhalation is an important form of drug abuse, estimated to have a lifetime prevalence of 18% in the United States. In this disorder, solvents are deliberately inhaled in high concentrations, often for months or years, to achieve a euphoric state. Toluene is the major solvent in spray paint and other readily available products. As a result of its lipophilicity, toluene readily enters the brain and may exert profound toxic effects. Chronic exposure to toluene produces a characteristic neurologic disorder in which dementia, ataxia, and other neurologic deficits can be prominent.
Methods: Our group has had the opportunity to study a number of individuals with long-term solvent vapor abuse in whom exposure to toluene has been heavy and prolonged. Clinical, neuropsychological, magnetic resonance imaging (MRI), and neuropathological data have been collected and analyzed.
Results: A series of 14 individuals was given a complete neurologic examination, comprehensive neuropsychological testing, and an MRI scan of the brain. There were 10 men and four women. The age range was 20-43 years, with a mean of 28. The duration of heavy exposure to toluene ranged from 24-252 months, with a mean of 105. Neurologic examinations were notable for various degrees of cognitive impairment, variable ataxia, and assorted brainstem signs; there was no evidence of peripheral nervous system involvement. The neu-

ropsychological and MRI data were reviewed blindly by investigators who did not know the results of the other evaluation. Neuropsychological testing revealed significant cognitive impairment, often clearly at a level consitent with dementia, in several individuals. The MRI scans often disclosed diffuse white matter hyperintensity on T2-weighted images and cerebral atrophy. The duration of toluene abuse correlated with the severity of cognitive impairment and the degree of cerebral white matter hyperintensity on MRI. In addition, one other individual with toluene abuse died, apparently from the effects of toluene on the myocardium, and the brain autopsy disclosed dramatic loss of myelin in the cerebral hemispheres and cerebellum with sparing of cell bodies and axons.

Discussion: Toluene is a neurotoxin with specific effects on the white matter of the brain. Myelin appears to be the major target. When individuals expose themselves to prolonged high levels of inhaled toluene, toluene leukoencephalopathy can develop and produce a devastating clinical picture. Our data suggest that the amount of exposure correlates with both the extent of white matter damage in the brain and the severity of cognitive impairment. The dementia that occurs in toluene leukoencephalopathy is a striking example of the neurobehavioral dysfunction that can result from damage to brain white matter. To highlight this unusual observation, we have chosen the descriptor white matter dementia, a term that can also be applied to other disorders of white matter that produce a similar syndrome. The precise threshold of toluene exposure at which brain damage occurs is difficult to determine based on the available clinical data, and these findings clearly apply to humans who have sustained massive exposure to the solvent. However, more detailed information is urgently needed because of the high prevalence of this type of substance abuse and the potential for more subtle forms of toluene leukoencephalopathy to occur in those with less intense exposure. Implications for exposure to toluene in the workplace and the home environment are also apparent. Toluene leukoencephalopathy demonstrates the remarkable capacity of toluene to damage the brain through selective toxicity to brain myelin, and more generally, the role of cerebral white matter in normal cognition.

Panel Session
Drug Addiction: A Disorder of Pathological Learning and Memory

Signal Transduction Pathways Used by Therapeutic Agents and Drugs of Abuse

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The concept that nerve cells communicate with each other through two distinct mechanisms, referred to as fast and slow synaptic transmission, is now well-established. A number of components of the two signal transduction pathways have been identified. Fast-acting neurotransmitters, e.g., glutamate (excitatory) and g-aminobutyric acid (GABA) (inhibitory), achieve effects on their target cells within one millisecond by virtue of opening ligand-operated ion channels. In contrast, all of the effects of the biogenic amine and peptide neurotransmitters, as well as many of the effects of glutamate and GABA, are achieved over hundreds of milliseconds to minutes by slow synaptic transmission. This latter process is mediated through an enormously more complicated sequence of biochemical steps, involving second messengers, protein kinases, and protein phosphatases. Slow-acting neurotransmitters control the efficacy of fast synaptic transmission by regulating the efficiency of neurotransmitter release from presynaptic terminals and by regulating the efficiency with which fast-acting neurotransmitters produce their effects on postsynaptic receptors. A growing body of knowledge concerning slow signal transduction pathways has been utilized to elucidate the mechanism of action of therapeutic agents used for the treatment of schizophrenia, Parkinsonism, and depression, as well as of drugs of abuse, such as caffeine, cannabis, amphetamine, PCP, and LSD.

Long-Term Plasticity in the Mesolimbic System and its Potential Role in Drug Addiction

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The neurobiological changes leading to behavioral sensitization to cocaine have been suggested to reflect drug-induced neuroplasticity in circuits that increase the incentive value of drugs. Previous studies have provided evidence for a central role of NMDAR in psychostimulant sensitization, and my laboratory has recently shown that cocaine-induced plasticity of glutamatergic synaptic transmission in the ventral tegmental area (VTA) plays an important role in brain adaptations that might promote addictive behaviors. However, the mechanism responsible for triggering these long-term synaptic changes is unknown. By using patch clamp recordings, my laboratory has collected evidence showing that cocaine caused a delayed increase in N-methyl-D-aspartate receptor (NMDAR)-mediated synaptic currents in putative VTA dopamine (DA) cells. This effect was mimicked by a specific DA re-uptake inhibitor and by a DA D1/D5 receptor agonist and blocked by a DA D1/D5 receptor antagonist. Further, biochemical analysis showed a redistribution of the NMDAR subunits NR1 and NR2B in VTA cell synaptic membranes. In agreement with these findings, NMDAR-mediated EPSC decay-time kinetics were significantly slower after cocaine, and pharmacological analysis indicates that NR2B subunits are not in the form of 'pure' NR1/NR2B complexes, but likely in the triheteromeric NR1/NR2A/NR2B form. Taken together, our data suggest that acute cocaine increases NMDAR function in the VTA via activation of cAMP/PKA pathway mediated by a DA D1/D5 receptor leading to the insertion of NR2B-containing NMDAR in the membrane. Based on this as well as on additional evidence collected from my laboratory, I will discuss a) the role of this acute increase of NMDA responses by cocaine in facilitating long-term plasticity and b) the relationship between long-term synaptic plasticity in the mesolimbic system and operant behaviors such as cocaine self-administration.

Drug-Induced Structural Plasticity

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Repeated exposure to drugs of abuse can produce many long-lasting changes in brain and behavior, some of which may contribute to the development of psychopathological states, including psychosis and addiction. There has been, therefore, considerable interest in identifying the nature of persistent drug-induced changes in neural circuits implicated in drug-associated psychopathology. This talk will review evidence that repeated exposure to drugs of abuse alters the structure of dendrites and dendritic spines in many different brain regions involved in incentive motivation, learning and cognitive function, including the nucleus accumbens, caudate-putamen, hippocampal formation, medial frontal cortex, orbital frontal cortex and other neocortical regions. However, the nature of drug-induced changes in dendritic structure varies greatly as a function of the drug administered (amphetamine, cocaine, nicotine or morphine), the amount of drug administered, the mode of drug administration (whether it is administered by an experimenter or self-administered), and the cell and portion of a dendritic tree examined. Furthermore, the effects of drugs interact with the effects that other kinds of experiences have on dendritic structure, and in ways that may limit the potential for other forms of experience-dependent plasticity. The picture that is emerging from these studies is one in which different drugs of abuse can reorganize patterns of synaptic connectivity in very specific ways, and in a regionally-specific manner, and by this means selectively influence the operation of diverse neural circuits. A challenge for the future will be to relate drug-induced changes in specific circuits to the operation of a circuit, and eventually to diverse changes in behavioral and cognitive functions mediated by the brain systems enduringly reorganized by drugs.

Human Genome Scanning Results Support Roles for Mnemonic Systems in Addiction Vulnerabilities

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Human addiction vulnerability association genome scanning results now implicate variants in a number of gene families in addiction mechanisms. There is a caveat: some genes whose products may be centrally involved in addiction mechanisms may not harbor common functional allelic variants that contribute to interindividual differences in addiction vulnerability. Nevertheless, assessing the kinds of genes detected by genome scanning data can provide information about human addiction mechanims. We summarize our data from four association genome scans in individuals who are dependent on legal and/or illegal substances vs matched controls. These data implicate many gene products that are strongly implicated in mnemonic processes for roles in addiction. We focus on genes related to "cell adhesion" mechanisms that play central roles in establishing and maintaining neuronal connectivities and contain reproducible addiction association genome scanning signals. Such results should add to the attention now being focused on the memonic features that are likely to underlie substantial aspects of human addictions.

Panel Session
Mechanistic Convergence of Cortical GABA and Glutamate Theories of Schizophrenia

Abnormalities of Gamma EEG Oscillations in Schizophrenia

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Background: Previously we have found abnormalities in the stimulus-evoked gamma-band (20-100 Hz) responses of schizophrenia patients, including a negative correlation between the frequency of a response and symptom severity. Here we examined gamma-band responses that were time-locked to individuals' reaction times in a Gestalt perception task. We hypothesized that such responses would be more closely related to feature-binding processes than stimulus-evoked responses, and hence would be more sensitive to the severity of schizophrenic symptoms.
Methods: Chronic medicated male schizophrenia patients (N=18) and matched healthy controls (N=18) discriminated between Gestalt (illusory square) and control patterns by pressing a button for each stimulus type. The EEG was sampled at 500 Hz from 16 scalp sites. Time-frequency decomposition of the EEG was performed using the Morlet wavelet. Single-trial epochs were aligned according to the reaction time on each trial. Phase-locking was computed across correct trials in each condition.
Results: In all subjects, Gestalt patterns evoked a phase-locked gamma-band response at posterior electrode sites. As this response was phase-locked to the reaction time, it may reflect visual feature-binding and/or the formation of a perceptual decision. The frequency of this response was significantly lower for schizophrenics (22-25 Hz) than controls (34-40 Hz), and was negatively correlated with hallucination severity (-.52, $p < .05$). Medication dosage was not correlated with the degree of oscillatory abnormality.
Conclusions: These data add to the growing evidence that gamma-band synchrony reflects neural circuit abnormalities in schizophrenia. In particular, the negative correlation between hallucination severity and synchronization frequency suggests that core symptoms of schizophrenia are related to the integrity of neural circuits. An In Vitro animal model of gamma band anomalies is compatible with abnormal glutamatergic-GABAergic neuronal interaction.

Regional Patterns of Cortical GABA Gene Expression in Schizophrenia: Mechanisms and Consequences

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Background: In the dorsolateral prefrontal cortex (DLPFC) of subjects with schizophrenia, the expression of certain GABA transcripts including the 67 kD form of glutamic acid decarboxylase (GAD67), a synthesizing enzyme for GABA, and the GABA membrane transporter (GAT1) are reduced in the subpopulation of GABA neurons that express the calcium-binding protein parvalbumin (PV). Given the role of networks of PV-positive GABA neurons in generating gamma oscillations, these impairments likely contribute to the reduced gamma power observed in the DLPFC of subjects with schizophrenia. However, whether similar alterations in PV neurons could account for altered gamma oscillations observed over other cortical regions has not been examined.
Methods: In order to characterize the regional patterns of GABA-related transcript expression in schizophrenia, we used quantitative RT-PCR to examine the relative tissue concentrations of 9 GABA transcripts and 3 normalizers across different cortical regions in subjects with schizophrenia and matched comparison subjects. In addition, in order to explore the mechanisms contributing to these gene expression changes, we examined several mouse genetic models.
Results: In the DLPFC, the normalized expression levels of 5 of the 9 GABA transcripts, including GAD67 and PV, were reduced in the subjects with schizophrenia, whereas the expression of other transcripts such as GAD65, was unchanged. Preliminary data from the anterior cingulate and primary visual cortices in the same subjects revealed similar patterns of change, with some regional differences in the relative magnitude and consistency of change across subjects. The potential pathogenetic mechanisms, including reduced signaling through NMDA receptors, that could confer this regionally conserved pattern of altered GABA gene expression are being evaluated in mouse genetic models.
Conclusion: These findings suggest that a conserved pattern of altered GABA neurotransmission is present across the neocortex in subjects with schizophrenia, and that these alterations could contribute to impairments in gamma oscillations under conditions that tap the function of different neocortical circuits.

Region-Specific Reduction in Entorhinal Gamma Oscillations and Parvalbumin-Immunoreactive Neurons in Animal Models of Psychiatric Illness

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Background: Psychiatric illnesses, particularly schizophrenia, are associated with disrupted markers for interneuronal function and interneuron mediated brain rhythms such as gamma frequency oscillations.
Methods: We investigated a possible link between these two observations in the entorhinal cortex and hippocampus by using a genetic (Lysophosphatidic acid 1 receptor deficient (LPA1-deficient) mouse) and an acute model (Ketamine) of psychiatric illness. Intra- and extracellular recordings from enthorinal and hippocampal slices were accompanied by immunocytochemistry for specific interneuronal markers (GABA, parvalbumin, calretinin).
Results: Lysophosphatidic acid 1 receptor deficient (LPA1-deficient) mice show psychomotor-gating deficits and neurochemical changes resembling those seen in postmortem schizophrenia studies. Similar deficits are seen acutely with antagonism of the NMDA subtype of glutamate receptor. Neither model induced any change in power or frequency of gamma rhythms generated by kainate in hippocampal slices. In contrast, a dramatic decrease in the power of gamma oscillations was seen in superficial, but not deep, medial entorhinal cortex layers in both

models. Immunolabeling for GABA, parvalbumin, and calretinin in medial entorhinal cortex from LPA1-deficient mice showed an 40% reduction in total GABA- and parvalbumin-containing neurons, but no change in the number of calretinin-positive neurons. This deficit was specific for layer II (LII). No change in the number of neurons positive for these markers was seen in the hippocampus. Acute NMDA receptor blockade with ketamine, which selectively reduces synaptic drive to LII entorhinal interneurons, also disrupted gamma rhythms in a similar manner in superficial entorhinal cortex, but not in hippocampus.
Discussion: These data demonstrate an area-specific deficit in gamma rhythmogenesis in animal models of psychiatric illness and suggest that loss, or reduction in function, of interneurons having a large NMDA receptor expression may underlie the network dysfunction that is seen.

Reduced NMDA Receptor Function Produces Disinhibition of Prefrontal Cortex Output Neurons: Correlation with Behavior and Reversal by Clozapine
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The computational integrity of the prefrontal cortex (PFC) is thought to be disrupted in schizophrenia. Many of the behavioral deficits associated with cortical dysfunction in schizophrenia are modeled in healthy individuals treated with NMDA receptor blockers. To define the mechanisms by which a general state of NMDA deficiency may lead to cortical dysfunction, we used ensemble recording in behaving rats. Systemic blockade of NMDA receptors, at doses that impaired working memory, potentiated the firing rate of prefrontal cortex neurons. This potentiation, which correlated with expression of behavioral stereotypy, resulted from an increased number of irregularly discharged single spikes. Clozapine but not haloperidol, produced an activity-dependent influence on spontaneous firing rate of PFC cells: it increased the activity of the neurons with low baseline firing rates and decreased the activity of neurons with higher firing rates. Clozapine but not haloperidol, also reversed the effect of NMDA receptor hypofunction on PFC neuronal firing. Novel ligands that enhance NMDA receptor currents through activation of metabotropic glutamate 5 receptors mimicked the effects of clozapine. The reversal by clozapine or glutamatergic ligands was strongly correlated with blockade of NMDA antagonist-induced behavioral disruptions. These findings suggest that at least some of the adverse effects of NMDA receptor hypofunction may arise from a disorganized overactivation (or disinhibition) of cortical neural ensembles. This may lead to improper sequencing of behavioral sets leading to motor and cognitive stereotypies. The fine tuning of these ensembles by clozapine would restore the PFC capability to choose the appropriate behavioral repertoire.

Panel Session
Molecular Libraries Roadmap: Small Molecules, Big Science

Discovery and Characterization of Novel Allosteric Modulators of GPCRs
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Background: Selective activators of specific subtypes of metabotropic glutamate receptors (mGluRs) have exciting potential for development of novel treatment strategies for psychiatric and neurological disorders. For instance, selective agonists of mGluR5, mGluR4, and mGluR2 could provide novel approaches to treatment of schizophrenia, Parkinson's disease, and anxiety disorders respectively. Unfortunately, it has been difficult to develop compounds that act as selective orthosteric (glutamate site) agonists of specific mGluR subtypes that have properties that are suitable for development of therapeutic agents. Similar difficulties have been encountered with other families of GPCRs. For instance, multiple groups have attempted to develop highly selective agonists of muscarinic receptors as a novel approach to treatment of Alzheimers disease. Availability of structurally diverse small molecule libraries along with technologies for high throughput screening, chemi informatics, and medicinal chemistry, have allowed us to focus on a new approach to activation of these important GPCR classes by targeting allosteric sites that potentiate receptor responses to activation by agonists.
Results: We and others have been highly successful in developing highly selective allosteric potentiators of specific mGluR subtypes. These compounds do not activate mGluRs directly but potentiate the response of these receptors to glutamate. These allosteric potentiators offer high selectivity for the targeted receptor and provide an exciting new approach to development of novel selective activators of these and other GPCR subtypes. Interestingly, we have found that multiple allosteric regulators appear to interact with similar binding pockets that are conserved between receptor subtypes. Furthermore, compounds can act at a single site to serve as allosteric potentiators, antagonists, or neutral ligands. However, multiple binding sites for allosteric regulators can also exist on a single receptor subtype. Interestingly, we have also found that allosteric potentiators can differentially regulate mGluR coupling to different signaling pathways or functional responses in different neuronal populations. Thus, these compounds may offer an opportunity to selectively modulate different responses to a single receptor subtype. We have now performed in vivo studies that reveal that these compounds have robust effects in animal models that have been used to predict efficacy for novel anxiolytics (mGluR2), antipsychotic agents (mGluR2, mGluR5), and antiparkinsonian agents (mGluR4). Most recently, we and others have completed high throughput screens and chemi informatic studies to discover novel highly selective allosteric potentiators for M1 and M4 muscarinic receptors. These studies suggest that this approach is not restricted to family C GPCRs but may also provide a viable approach for discovery of selective activators for members of other GPCR families.
Discussion: These studies provide an exciting new approach to discovery of novel highly selective activators of specific GPCR subtypes. In addition to providing unprecedented selectivity, these compounds may have other properties that are preferable to traditional agonists, such as reduced receptor desensitization and adverse effects due to maintenance of activity dependence of receptor activation. This provides an excellent example of the power of HTS, chemi informatics and medicinal chemistry to discovery molecules that could not be predicted based on protein structure or previously known chemical scaffolds.

Novel Targets for the NIH Roadman Molecular Libraries Initiative
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The high throughput (HT) flow cytometry platform HyperCyt is adept at both cell and particle-based assays and is compatible with both high content and multiplex analysis. Cell-based assays have initially been directed against G protein-coupled receptor (GPCR) targets where we have identified novel small molecule ligands for the GPR30 membrane estrogen receptor that discriminate it from the classic nuclear ER alpha and ER beta receptors. Derivatives of these small molecules are expected to be useful in fluorescence and isotopic imaging studies. We have developed general particle-based multiplexed approaches compatible with assemblies of soluble membrane receptors, receptor tails, proteases, kinases, nucleases, etc. We have probed the mechanism of partial agonism of the beta2-adrenergic receptor and the steps in signal transduction using flow cytometry-based kinetic analysis with soluble GPCR. The NIH Roadmap Molecular Libraries Initiative (MLI) has given us the opportunity, through

the New Mexico Molecular Libraries Screening Center (NMMLSCN, http://nmmlsc/) to implement HT flow cytometry for the international research community. Through MLI and collaborations with investigators outside the MLSCN, we are currently developing a wide variety of cell and molecular targets.

The NIH Molecular Libraries Initiative: Chemical Tools for the Neuropsychiatry in the Genome Era

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The NIH Chemical Genomics Center (NCGC, http://www.ncgc.nih.gov) is an ultrahigh-throughput screening and chemistry center which discovers chemical probes of gene and cell functions across the genome using its quantitative HTS (qHTS) technology, and develops new paradigms for screening that enable chemical genomics and downstream drug development. Located within the National Human Genome Research Institute as part of the NIH Roadmap Molecular Libraries Screening Center Network (MLSCN), all of the data produced at the NCGC are made freely available via PubChem (http://pubchem.ncbi.nlm.nih.gov). The products of the NCGC are novel chemical probe series with activity, potency, and solubility adequate for in vitro study of gene, protein, pathway, and cell functions in health and disease. Several assays relevant to orphan neuropsychiatric disorders are being screened at the NCGC and will be discussed, including those for glucocerebrosidase (the enzyme mutated in Gaucher's Disease), Spinal Muscular Atrophy, and Huntington's Disease.

Assay Design for Neuroscience Targets

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Near completion of the human genome sequence and other recent technology advances have created a situation in which the number of known proteins of neuropsychiatric interest has far outstripped our ability to manipulate the function of these proteins with genetics or small molecules. The vision of the Molecular Libraries and Screening Center Network, an NIH Roadmap Initiative, is to bring together chemists and biomedical scientists in an environment that facilitates the discovery and study of novel small molecules as both research tools and potential leads for therapeutic development. This talk will address how individual investigators can utilize this new opportunity to advance their research goals. Examples will be provided from Emory University laboratories, focusing on the development of small molecules that act on specific prostanoid receptors to blunt neuroinflammation. Because G protein coupled receptors represent the largest target class for marketed drugs, many assays have been developed that are suitable for primary HTS. Assays for cAMP accumulation or IP3-mediated Ca2+ imaging have been developed based on scintillation proximity, fluorescence polarization (FP), time-resolved fluorescence FRET, amplified luminescence, enzyme complementation, electroluminescence, and fluorescence intensity. Cell-based assays have been developed to quantify the translocation of fluorescent proteins from one cellular compartment to another, the clustering or internalization of cell surface receptors, or morphometric measurements such as neurite outgrowth. Whole animal assays involving zebrafish are also receiving a good deal of attention. The key challenge in each case is to convert a target or phenotypic assay from benchtop to robot. Adaptation of a successful bench assay to high throughput mode requires optimizing the robustness and stability of the assay readouts, taking cost per well including labor costs into consideration. The process typically begins by optimizing the concentration of protein and substrate in each well for a biochemical assay, or the number of cells per well in the case of cell-based assays. The parameters to optimize are cost of reagents, the signal to background ratio (S:B), and the Z' factor. The Z' factor measures the quality of the assay itself without intervention of test compounds. It is calculated from the following equation. Z' factor = 1 – (3*SDs + 3*SDb) / (Ms – Mb) Where the s subscript refers to the maximum assay signal (eg in the presence of a screening concentration of agonist), b the minimum signal (eg, in the absence of agonist), and M the mean signals in each condition. S:B >5 and Z'>0.5 are typical criteria that portend an assay robust enough for HTS. The next step in assay optimization is to determine whether interplate and intraplate variability are low enough, and whether 1-2% DMSO, the most common vehicle for small molecule library compounds, adversely affects assay robustness. Finally, it is essential to show that a positive control affects the assay readout in a stable manner over the expected test period. In the first year of the MLSCN, neuroscience targets have represented approximately one third of all targets selected for screening, and cell-based assays well over half of the total. This NIH-supported national resource should continue to add value to neuropsychiatric research programs.

Panel Session
Neuroimaging and Genetics Across the Lifespan in Health and Illness

Early Brain Development Assessed by Quantitative Analysis of Structural MRI and DTI

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Background: Imaging studies of early brain development get increasing attention as improved modeling of the pattern of normal development might lead to a better understanding of origin, timing and nature of morphologic differences in neurodevelopmental disorders. We have collected one of the largest samples of MRI/DTI of neonates and infants of ages 1yr, 2years and 4years to model the trajectory of early brain development using structural MRI and diffusion tensor imaging (DTI). Studying this age group involves two major challenges, successful MRI scanning of non-sedated infants and new image analysis methods that can cope with low contrast-to-noise ratio, variability of brain shape and size, and locally varying contrast changes due to early myelination.
Methods: Tissue segmentation and lobe parcellation: We have developed a new fully automatic brain segmentation and tissue segmentation technique that uses probabilistic brain atlases (neonate, 2yrs, 4yrs) as spatial priors. The tool incorporates brain stripping, bias correction, atlas-registration, multi-channel MRI registration, and probabilistic segmentation including separation of myelinated and non-myelinated white matter into one package. Computational anatomy tools for building spatio-temporal 4-D atlases of brain growth: We have developed a new computational anatomy tool is based on the concept of unbiased atlas building. A group of 3-D images is simultaneously deformed to build a new average center image, which is a sharp MR image encoding the average structures of the whole population. Group differences and longitudinal change is assessed by quantifying local deformation between pairs of atlases. This new method provides a fully volumetric description of the growth pattern. White matter development based on DTI: Local diffusion properties in white matter are associated with axon density, degree of myelination and density of white matter. We developed new tools for precise nonlinear inter-subject registration of DTI maps and quantitative tractography to delineate and quantify properties of major fiber tracts.
Results: Method developments are driven by the needs of several clinical pediatric studies at UNC Chapel Hill. This includes a longitudinal study of neonatal brain development in high risk children and controls (N total =134), with follow-up at 1 year of age, a neonate twin MRI study, and an autism study (51 autistic (AUT) and 25 control individuals (TYP, DD)) with baseline scans at 2 years and follow-up at 4 years). Structural MRI reveals a quantification of re-

gions of early myelination of the projection tract up to the motor cortex, while also providing high-quality segmentation of non-myelinated white, gray and fluid volumes. Early growth is mostly accounted for by a rapid change of cortical gray matter. The most striking result of the longitudinal growth analysis between 2 and 4 years is the apparent cerebral asymmetry. There is a consistent right frontal > left frontal and a left posterior parietal/occipital > right posterior parietal/occipital pattern, commonly called torque or brain torsion.

Discussion: Measuring the trajectory of growth via structural MRI and DTI will likely provide a vastly improved understanding of early brain development, changes due to delayed development or pathology, and its relationship to neuropsychiatric disorders. Our preliminary findings indicate that the new methodology shows excellent potential to explore longitudinal change, difference between groups, and differences between growth trajectories between groups.

Longitudinal Trajectories of Brain Development in Healthy Children and Adolescents

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Background: Differences in cognition, behavior, and emotions between children, adolescents, and adults have been noted for millennia. Characterizing the neuroanatomical substrates of these differences has been more elusive. In 1989, the Child Psychiatry Branch at the National Institute of Mental Health (NIMH) initiated the first large scale longitudinal study of normal and abnormal brain development. This presentation will summarize findings related to normal brain development and factors related to sex, genes, and environment that affect it.

Methods: Subjects are recruited from the community and undergo physical and neurological exams, clinical interviews, family history assessment, and an extensive neuropsychological battery. Participants are asked to return for follow-up longitudinal testing and scans at approximately 2 year intervals. As of December 2005 the data set included over 1000 MRI scans obtained from several hundred healthy children. A variety of analysis techniques have been used through collaborations with imaging centers including UCLA, the Montreal Neurological Institute, and the University of Iowa, with an emphasis on automated methods due to the large volume of scans in the dataset.

Results: Cortical and subcortical gray matter volumes exhibited an inverted U shaped trajectory which was robustly sexually dimorphic in nearly all structures. Total volume in girls generally peaked earlier than in boys. Total white matter volume increases with age in both sexes. The gray and white matter of the cerebellum shows similar trajectories to that in the cerebrum. The trajectory of the vermis is markedly different than that of the cerebellar lobes. Using novel cortical pattern matching algorithms, we created dynamic maps of healthy brain development from early childhood through late adolescence, which showed that structural cortical maturation follows functionally relevant milestones. We have also compared cortical thickness between males and females, finding that the trajectories of development of cortical thickness differ between males and females in a regionally heterogeneous fashion. Measures of brain lobar volumes and cortical thickness were analyzed in an extended twin study design using structural equation modelling. Findings included high heritability of lobar brain volumes, consistent with previously reported studies in adults. Intriguingly, the proportion of variance due to genetic factors appears to decrease in gray matter while it increases in white matter over the age range studied. Analysis of heritability of cortical thickness measures in this pediatric twin population found higher heritability measures in the frontal, superior parietal, and superior temporal regions, with significant asymmetry of regions of high heritability between the hemispheres.

The potential functional relevance of brain trajectories was demonstrated in a comparison of brain trajectories in children with average, high, and very high IQ scores. The children in the group with the highest IQs were found to have a more dynamic brain growth pattern than the other two groups.

Conclusion: The past decade has seen an increasing appreciation of the prolonged postnatal development of the human brain and the importance of neurodevelopmental factors in the pathogenesis of many common psychiatric disorders. Understanding the course of brain growth in healthy children and what can affect it for good or ill is a crucial element in the effort to better elucidate these processes and to target interventions to optimize outcome.

Brain Development in Children and Adolescents with Psychotic Disorders: Insights from Longitudinal Neuroimaging and Genetic Studies

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We have been following the course of brain development in children and adolescents with psychotic disorders for over fifteen years using longitudinally acquired anatomic brain MRI scans. The clinical populations studied include children with childhood onset schizophrenia (COS), bipolar illness and atypical psychosis. Using novel cortical pattern matching algorithms, we have been able to compare cortical development in these children with similarly constructed time lapse sequences in normally developing children. These dynamic maps reveal significantly distinct patterns of brain development for these populations, confirming that these illnesses are neurobiologically distinct. Specifically, brain development for COS shows a 'back to front wave' of profound GM loss during adolescence evolving into the adult pattern by age 25. On the other hand, cortical development in pediatric bipolar illness (mapped before and after the onset of bipolar illness), shows a subtle pattern of GM gain in left temporal cortex and GM loss in cingulate areas, which also overlaps with that seen in atypical psychosis, and is distinct from that seen in COS. Our recent studies show that the cortical GM development in schizophrenia could be a train marker (endophenotype), as suggested by our cortical thickness analyses on longitudinally studied healthy siblings (n=52, 110 scans) of COS patients. These siblings, who have no schizophrenia spectrum diagnoses and no medication exposure, show a similar pattern of GM deficits mainly localized to the prefrontal and temporal cortices. However, the GM deficits in healthy siblings appear to normalize by the time the siblings are 20 years old, also following a parieto-frontal-temporal pattern that seen in the COS probands suggesting that the GM developmental trajectories (not just the deficits) could be an important endophenotype to study. We are currently evaluating the effects of the risk alleles on quantitative measures of brain morphometry, using a recently developed fully automated cortical thickness analysis technique that can rapidly process much larger samples. Our recent analyses analyses show that both COS probands and their healthy siblings have steeper slopes of frontal GM loss when they have the risk allele for GAD (a GABA modulator enzyme) gene. Moreover, in healthy COS siblings the initially decreased GM appears to normalize with age suggesting a genetically influenced restitutive process. Results from analyses for other recently confirmed risk genes for our COS sample will also be presented.

Dynamics of Brain Changes in Alzheimer's Disease Mapped with a Population Based Brain Atlas

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Due to recent innovations in brain mapping, the dynamic spread of Alzheimer's disease can now be charted in the living brain. Effects of drug treatment, risk genes, and brain deficits can be tracked as they emerge, in patients and those at genetic risk. These maps can be warehoused in statistical brain atlases, which encode dynamic and enetic information on brain change in entire populations, across the human

lifespan. We show how these tools help to explore and map the disease process, revealing factors that affect it. Novel mathematics are described for visualizing therapeutic and gene effects. Dynamic Spread of AD: As an illustrative example, we report the mapping of a dynamically spreading wave of gray matter loss in the brains of Alzheimer's patients, scanned repeatedly with MRI. The loss pattern is visualized, in 3D video data, as it spreads from temporal cortices into frontal and cingulate brain regions as the disease progresses. Deficit patterns are resolved with a novel 4D cortical pattern matching strategy, which resolves the dynamic path of the disease as it spreads in the human cortex over a period of several years. These dynamic sequences show a rapidly advancing wave of cortical atrophy sweeping from limbic and temporal cortices into higher-order association and ultimately primary sensorimotor areas, in a pattern that correlates with cognitive decline. A complementary technique, tensor-based morphometry,reveals the 3D profile of atrophic rates, at each point in the brain. Both techniques are highly automated, have been validated on thousands of scans, and are sensitive to clinically relevant changes in individual patients and groups undergoing different drug treatments. Time-Lapse Maps of Disease: 3D animations are shown that statistically compare time-lapse maps of different dementias, relate these changes to changes that occur normally with aging, and relate them to similar time-lapse maps of childhood development, schizophrenia, and HIV-associated dementia (Gogtay et al., PNAS, 2004; Thompson et al.,PNAS, 2001, 2005). We also report preliminary data mapping the dynamic trajectory of neurofibrillary and amyloid pathology in Alzheimer's disease with [18F]-FDDNP, a novel PET radioligand developed at UCLA (G. Small et al., 2006). Animations show how this marker of disease burden spreads dynamically in the brain, and how this trajectory predicts cognitive deterioration in normal elderly subjects. These video maps chart the dynamic progress of Alzheimer's disease. They reveal a changing pattern of deficits. We are now using them urgently to detect where deficit patterns are modified by drug treatment and known risk genotypes. Strengths and weaknesses of these different imaging biomarkers for drug trials and basic neuroscience studies are discussed.

Panel Session
Psychotropic Treatment During Pregnancy: Doing Good or Harm (or Both) on Which and Whose Outcomes?

Surveillance for Drug-Associated Adverse Reproductive Outcomes: Model Systems and Implications for Psychotropics

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Evaluations of potential drug-associated pregnancy risks are most often based on preclinical animal reproductive and developmental toxicity studies; however, such studies are not always predictive of human pregnancy outcomes. Furthermore, human pregnancy safety data are either inadequate or nonexistent for more than 80% of drugs currently marketed in the U.S. This issue is especially critical for women of reproductive age who require treatment with psychotropics, both because of the relatively high prevalence of psychiatric disorders among women in this age group, and because of the likely need for chronic treatment that may extend throughout pregnancy. Several research methods are utilized in the post-marketing arena to assess drug-associated adverse reproductive outcomes, including case series, pregnancy exposure registries, cohort studies, case-control surveillance studies, and large linked database studies. Each of these methods has strengths and weaknesses, and no single study design is adequate to comprehensively assess drug safety. This suggests that systematic combinations of methodologic designs are necessary to appropriately evaluate human pregnancy risk. Applying lessons learned from the anticonvulsant literature, a rational approach to a model system for evaluating adverse reproductive outcomes associated with psychotropic medications can be described, utilizing and expanding upon the strengths of existing resources.

Where Does Neonatal Syndrome Stop and Neurobehavioral Teratogenicity Begin?

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The study of infant behaviors after in utero antidepressant exposure is a field that is rapidly growing. In the recent two years, there has been great attention focused on a neonatal behavioral syndrome associated with in utero serotonin reuptake inhibitors (SRI: includes selective serotonin reuptake inhibitors [SSRI] and serotonin norepinephrine reuptake inhibitors [SNRI]). A review of the case literature, extended case series, and cohort studies indicates that the most common neonatal signs include jitteriness, increased muscle tone, feeding disturbances, and agitation. Cohort studies find that 30% of SRI-exposed neonates undergo a behavioral syndrome. The syndrome is mild and self limited, with approximately 1% or fewer cases reaching high severity. Supportive management in special care nurseries is typically the only intervention. Mechanisms of the SRI-related neonatal syndrome include toxicity (in fetuses that cannot eliminate the drug/drug metabolite rapidly) and withdrawal (in cases such as paroxetine exposure, where rapid rebound of cholinergic drive may be causative). On the other hand, neurobehavioral teratogenicity encompasses neonatal/infant/child developmental changes that may emerge early in life and are persistent over time. For example, studies of pain threshold increases in SRI-exposed infants are documented to persist beyond the period in which the SRI-related neonatal syndrome ends. Standard developmental tests (ie: Bayley) may lack the ability to discriminate developmental endpoints in infants with differing in-utero drug exposures. Methodologies to test for early and persistent behavioral changes are under development. An important consideration in the evaluation of both neonatal behavioral syndrome and neurobehavioral teratogenicity is the maternal milieu. Like SRI-exposure, maternal mental illness is an exposure that has been associated with neonatal agitation, altered muscle tone, and lethargy. Future reports of SRI-related neonatal behavioral syndrome must characterize maternal psychiatric symptoms. In addition, objective measures of medication exposure during late pregnancy and the early puerperium both provide assurance of the drug exposure and can assist in ascertainment of a withdrawal versus toxicity mechanism in the SRI-related neonatal behavioral syndrome. Neonatal signs and developmental assessments should be measured serially by evaluators that are blind to maternal diagnosis and maternal use of SRI.

Validation and Quantification of Fetal Exposure

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A variety of data bases, registries, and investigations have gathered information about the impact of maternal antidepressant use during pregnancy on obstetrical, neonatal, and infant outcome. Several factors limit definitive conclusions regarding either risk or safety of 'in utero' antidepressant exposure. The most obvious is a lack of confirmation of fetal exposure to the medication. Data derived from a Specialized Center of Research employing a prospective study (n=292) of placental passage – 11% of women did not take their medication. In women taking medication, the umbilical cord concentration at delivery is typically >60% of the maternal serum concentration raising questions about the feasibility of early neonatal withdrawal symptoms. Similarly, the majority of antidepressant outcome studies have required statistical control for tobacco, alcohol, and drug exposure in the antidepressant group. Again, this relies on self report and does not include other potential exposures. A history of alcohol abuse/dependence significantly increased exposure to other prescription and over the counter medications. Urine drug screen identified 15 women (5% of sample) who denied using other drugs were taking illicit drugs and/or prescription medications with abuse potential (e.g.

opiate, benzodiazepine), urine cotinine measures are pending. Another aspect of fetal exposure that could influence outcome is maternal depression/stress. Postpartum follow up at 6 months of these prospectively followed women, demonstrated that the accuracy of retrospective recall of active depression during pregnancy was limited, with 39% (69/174) being incorrect when using a BDI >10. The limitations of studies without confirming fetal exposure is not only germane to assigning potential risk, but also important in the failure to assign risk. Potential methods for improving confirmation of compliance in large data sets and the potential impact of these findings on the interpretation and application of outcome data from other sources will be discussed.

So What Do I Say to the Pregnant Woman in My Office Today?

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The FDA recommended a drug class labeling change to include information about neonatal complications associated with late pregnancy exposure to SSRI medications, as well as a consideration of drug taper near term. The opportunity to study women's choices to taper or remain on the same medication dose was provided by my NIMH funded study of antidepressant use during pregnancy. At 38 weeks gestation, 35 women (medicated sample at present) decided to either taper the drug before the due date (for all SSRI except fluoxetine) or stop the drug (fluoxetine). The drug was restarted on the day of delivery for all women who tapered pre-partum. A semistructured interview to obtain choice and rationale was performed. Decisions of women about drug management near term included: Taper drug, N=10, 29%; Continue dose, N=21, 60%; Delivered <38 weeks gestation, N=4, 11%. Several points can be made about this process: 1. For women who chose stop/taper drug prepartum, the reason given was to avoid neonatal signs associated with antidepressants. These were also women who were asymptomatic during late pregnancy. Historically, they discontinued the drug with a period of wellness prior to recurrence and had confidence that they could tolerate a two week period without medication. 2. The majority of women (60% of sample) chose to continue the same dose near term. This subset of women were those who were concerned about depressive symptoms and their ability to tolerate labor, delivery, and the postpartum period. These women often had developed worsening depression in the second and third trimesters. Many required dose elevations to sustain remission, as has been described in the literature on drug dosing during pregnancy. Two women were concerned about withdrawal symptoms in themselves with a short-duration taper before term. Several women had pregnancy complications and decided that tapering would be too stressful or would aggravate the obstetrical condition (preeclampsia, hypertension, gestational diabetes). The majority of women who remained on their medication through term asked for more details about the neonatal syndrome, particularly the duration, potential severity, and long-term effects. Several women asked about techniques for calming the infants from the study pediatrician to prepare for this possibility. The pediatrician uses techniques such as kangaroo care and calming through swaddling, sucking and sound and motion maneuvers. 3. Delivery prior to our planned taper occurred in four women, who therefore took the drug through delivery. Although an earlier taper could be elected, the longer the period off drug, the more likely symptom breakthrough will occur. The criteria that these women used to make choices about continuing or tapering their medication pre-partum were instructive and reasonable. Continued research on the assessment of the outcomes for mothers and newborns when women elect to either taper or continue will add to the sophistication of this particular risk-benefit decision process.